

**BIOCHEMICAL GENETICS
OF
SELECTED COMMERCIALY IMPORTANT
PENAEID PRAWNS**

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OF THE
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By

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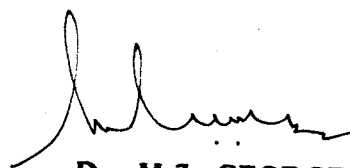


**CENTRE OF ADVANCED STUDIES IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
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[P. PHILIP SAMUEL]

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PREFACE

Production of fishes and crustaceans through natural resources is on the decrease in several countries, especially in India. Mariculture is therefore, a fast developing field, in fisheries, in view of both decrease in natural production as well as the enhancing demand of cheaper protein resources to be produced with scientific manipulation methods to bring about large scale production. It has gained momentum in all the developed and developing maritime countries. Especially in India, Crustaceans, Molluscs, fin fishes and seaweeds are the major important fields where much importance is given to improve the maximum return by culture methods. Keeping all this in mind the Central Marine Fisheries Research Institute (CMFRI), has taken up multidisciplinary programmes under the centre of advanced studies (CAS) in Mariculture of CMFRI funded by ICAR/UNDP/FAO Project.

After attaining M.Sc. degree in Zoology from the Madurai Kamaraj University in 1983 I joined in CAS in Mariculture as a Senior Research Fellow in the Ph.D. Programme in March 1984. During the first semester took up course work in Mariculture with a curriculum including

fishery and biological aspects of finfishes and shellfishes, culture methods of finfishes, prawn, lobster, crab, mussel, oyster, pearl, clam and sea weed along with site selection grow-out systems, production, economics and extension and environmental aspects.

Besides theory and practicals, study tours were undertaken to different Mariculture field laboratories of CMFRI. During the second semester a special subject "Fin fish and Shell Fish Genetics" was assigned for detailed study and I passed the Ph.D. qualifying examination conducted by the Cochin University of Science and Technology.

Afterwards the particular research project entitled "Biochemical genetics of selected commercially important penaeid prawns" allotted was carried out by collecting samples from different important fishing centres of India and the practical work was carried out in the Research Centre of CMFRI laboratories attached with those places. On the whole, in crustacea little importance has been given so far in finding out the genetic characteristics of different species, genetic variation within and between species and ontogenetic variations in lobsters, prawns and other crustaceans. Prawn is commercially important group

where very little attention had been given so far to find out the racial divergence which may exist in different species. With the increased foreign exchange earning and consequent indiscriminate over exploitation of existing resources of prawns resulting in depletion of the marine resources, alternative ways and augmenting production has become essential. In this connection genetic manipulation of the broodstock will surely bring about the heterogeneous characters to multiply production. In order to understand racial fragmentation of some of the commercially important prawns such as Penaeus indicus and Parapenaeopsis stylifera the isozyme studies were carried out. Ontogenetic variation of P. indicus showed stage specific electrophoretic variation. Inter species variation studies was carried out for the closely aligned Penaeus species like P. merguensis and P. penicillatus, P. japonicus, P. canaliculatus and P. latisulcatus. Metapenaeus sp. like M. brevicornis, M. affinis, M. monoceros and M. kutchensis, Parapenaeopsis species like P. stylifera, P. sculptilis and P. hardwickii.

These studies on inter species and intraspecies genetic variation along with morphometric variables and ontogenic genetic delineations carried out for the first time on Indian species of prawn would go a long way in delineating stocks in commercial populations and determining their genetic characteristics in order to use them for genetic engineering and

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CHAPTER I

INTRODUCTION

In an estimated total marine fish production of 1.61 million tonnes in India during 1984-85, prawns constituted 2,04,497 tonnes. Apart from this, according to a survey conducted in 1985 the total area utilized at present for prawn farming is 42,650 ha with a total production of 21,119 tonnes (Rao 1987). From all these, the foreign exchange earning for the country by export of marine products contributed mostly by prawn products is Rs. 460.67 crores in the year 1986-87. This would indicate the importance of prawns in the economy of the country.

Shrimp resources along the 6100 Km long coast line of India are exploited by both artisanal as well as mechanised sectors. The existing stock within the 30 m depth zone along the different regions of the coast is subjected to much pressure due to additional efforts by various programmes of mechanisation of the boats and motorisation of country crafts being implemented by many maritime states and also by the attraction and entry of big business people into the field. The shrimp production

which reached the maximum of 220,000 tonnes in 1975 showed a downward trend in subsequent years. This declining trend is seen in the recent years also. This naturally points towards the necessity for proper management of the fishery. In this context study of biological features of the fishery from different aspects has become quite essential.

The penaeid shrimp resources of the country which constitutes the coastal shrimp fishery occupy different ecosystems such as estuaries, inshore and offshore waters having, different environments. So the fishery management requires proper study of various biological aspects of the fishery in these different environments. Added to these the coastal shrimp fishery is multispecies fishery with individual species having its own distribution patterns, sizes and breeding activities (Silas, George and Jacob 1984). This situation requires monitoring of the population characteristics of each species separately to keep track of the effects of exploitation of the stocks.

Biology of economically important species of shrimp of the different regions of India are well

documented. Full bibliographies and reviews of the main features of shrimp biology are available in species synopsis papers and other publications by George (1970a, 1970b, 1970c, 1970d, 1972, 1978) Kunju (1970), Mohammed (1970a, 1970b, 1973), Rao (1970, 1973), Kurian and Sebastian (1975) and others. The important species contributing to the fishery are Penaeus indicus, Milne Edwards, P. monodon Fabricius, P. semisulcatus, De Haan, P. merguensis De Man, Metapenaeus dobsoni (Miers) M. monoceros (Fabricius), M. affinis (Milne Edwards) M. brevicornis, (Milne Edwards) Parapenaeopsis stylifera (Milne Edwards), P. sculptilis (Heller) and P. hardwickii (Miers). Various aspects like distribution different stages of life history reproduction, spawning, larval history and adult history of most of these species are known.

Among other features, delineation of stock and population structure of each species remains important in fisheries management and aquaculture (Hedgecock et al, 1977, Ihssen et al, 1981, Wilkins, 1981). Mark recovery experiments conducted by CMFRI (Vijayaraghavan et al, 1982) showed that P. indicus migrates from Cochin to South east

coast. This further complicates the delineation of stocks of this prawn in the fishery at different places. At the same time proper understanding of the stock contributing to the fishery is very essential for the management of the fishery.

In aquaculture a life is closed and cultivated in a controlled environment. This will result in domestication of an animal due to shielding of that animal from unfavourable environmental condition and long term genetic adaptation to an artificial environment. (Doyle and Hunte 1981). This may result in an ever increasing divergence between domesticated stocks and wild populations due to reduction of variability. In this situation details about the genetics of changes in fitness of each cultivable species is urgently required, since more and more prawn species will be brought under cultivation. These little known effects of aquaculture and similar fields of fisheries activities such as breeding and hybridization on existing species and their populations can be best evaluated and managed only if the existing species and their population structures are known at molecular level of organisation which is most natural.

Recently electrophoresis has gained acceptance in the problem of stock delineation (Saila and Flowers 1969, Messiah and Tibbo 1971, Parsons and Hodder 1971, Johnson et al, 1972, 1973, 1974; Messiah 1975, Smith et al, 1980, Lindsey 1981, Mulley and Letter 1981a, 1981b). However, very little work has been done in India to determine to what extent the prawn stocks differ genetically along their spatial range of distribution. Electrophoretic studies on planktonic juveniles and adults of P. indicus and P. monodon has been done by Sriraman et al, (1977). Protein patterns of different tissues of M. affinis, M. monoceros, P. hardwickii and P. stylifera has been studied by Kulkarni (1980). Thomas (1981) has shown the structure of different fraction of the muscle of P. indicus, M. dobsoni, M. monoceros and M. affinis. Different proteins of tissues specific and species specific pattern of P. monodon was found out by Prathibha (1984).

The genetic structure of most economically important fish and shrimp populations still remains unknown due to the absence of gene frequency data. These populations have been the object of intense fisheries research due to relative importance of natural selection and random forces

on the pattern of genetic variation. Species can be subdivided into genetically differentiated populations. Constituent populations in a mixed population have to be traced out. Knowledge of stock composition is the fundamental tool for effective management on mixed stock fisheries (Larkin 1981). Absence of this knowledge will result in over exploitation. Patterns of gene flow within each species of rare alleles are also important. Rare alleles can be used in genetic tagging or marking of fish stocks (Mangaly and Jamieson 1978, Lester 1979). By specific pattern of enzymes a key can be produced in solving some identification problems (Johnson et al, 1974). By the paternal protein pattern the hybrid can be identified. Enhancement of inbreeding effect can be identified by the homozygosity estimation.

For the conservation of genetic resources, the United Nations Environment Programme has recommended consultation with experts for conservation techniques of the fish genetic resources, to establish a mechanism for monitoring changes in the genic diversity of fish production, to produce a catalogue of genetic material, to promote knowledge of fish genetics to enhance genetic

diversity and to promote the management of ecosystem with rich genetic diversity for a major socio-economic role. FAO has recommended to conserve genetic resources of fishes in man made or natural ecosystem and to have sample population in the genetic resources centre or in the form of gene pool of gamete storage and germ plasma banks.

In India the National Bureau of Fish Genetic resources Institute has been initiated with the aim of collection and classification of information of genetic resources, to maintain fish genetic material, introduction of new species and conservation of endangered species. Its main thrust is to find the ecological and taxonomic survey of natural habitats, to identify genetically distinct populations with advanced technique, cataloguing the genotype, developing methods to conserve exploited and endangered species.

Ontogenetic variations can be used in identification of different stages of a species with their characteristic protein fractions.

Considering the importance of these studies a detailed morphometric and electrophoretic investigation

was carried out for the separation of populations of two different species of commercially important penaeid prawns Penaeus indicus and Parapenaeopsis stylifera which occur along the Indian coast. The phenotype and genotype difference which may exist between populations were investigated by the studies of gene enzyme variation in natural populations of the two commercially important penaeid prawns (Penaeus indicus and Parapenaeopsis stylifera) This will quantify the amount of racial divergence, if any, among geographically separated natural populations. Thus subpopulation differences within each species of penaeid prawn can be elucidated.

In the biochemical genetic studies electrophoresis is a promising technique for the detection of individual protein variants on gel media such as starch polyacrylamide and agar coupled with histochemical staining procedures.

In 1807 the principle of electrophoresis was found out by Alexander Reuss a Russian physicist. When electricity was passed through a glass tube containing water and clay, colloidal particles moved towards the positive electrode. Tiselius (1937) cited by Brewer (1970) was the first to do the moving boundary electrophoresis and thus separated serum proteins using electric current in a solution. Subsequently zone electrophoresis was developed and the protein

was separated in a stabilized media rather than a solution. Other methods developed by crustacean workers include paper, (Hughes and Klinkler, 1966) agar gel, (Decleir 1961) cellulose acetate, (Lim and Lee 1970) Starch gel, (Whittaker 1959 Cowden and Coleman 1962) and polyacrylamide gel electrophoresis (Dall, 1974, Alikhan and Akthar 1980).

In the present study polyacrylamide gel electrophoresis, having the following advantages was used. Sieving process in acrylamide can be adjusted by a varying proportion of cross linkage, by the addition of a proportion of bisacrylamide before polymerization. The bands formed by the larger proteins in acrylamide gels are considerably sharper than those of the same protein in starch gel. Acrylamide has an uncharged matrix in which separation is based on molecular sieving and mobility difference. But in starch proportion of COOH - group at neutral pH carry negative charge (Gordon 1978).

Genetic basis of electrophoretic variation is based on the known relationship between gene and structural protein band detected on the polyacrylamide gel (Crick 1963; Nirenberg et al, 1963, Ochoa 1963). First the sub-unit composition and structural relationship of the isozymes were studied in the individual species.

Secondly this isozyme technique was studied on different populations to understand population genetics of a particular species. Detailed work of allozymic variation between different population of P. indicus collected in Cochin, Tuticorin, Madras and Waltair and P. stylifera collected in Cochin and Bombay was carried out in addition to ontogenetic variation in P. indicus.

General protein differences of closely allied species like Metapenaeus brevicornis, M. affinis, M. kutchensis and M. monoceros, Parapenaeopsis hardwickii, P. stylifera and P. sculptilis, Penaeus latisulcatus, P. japonicus and P. canaliculatus, P. penicillatus and P. merguensis were also studied in detail for detecting species specific genetic characteristics.

The results of these studies would give the necessary scientific and natural basis for the species verification and their genetically differentiated populations if any. Gene flow within each species also can be identified. This will be helpful to find out the rare alleles in the population, which will act as the genetic tag and also an indication of the movement of larval and adult prawns between areas.

Here electrophoresis of different enzyme protein has been adapted as an effective tool to quantify the amount of racial divergence among geographically separated natural populations of P. indicus and P. stylifera. This gives an insight for its taxonomic information by determining its degree of protein divergence between species and specimens of the same species from different populations where the identification is not clear and many of the discriminate quantitative characters overlap.

Genetic characterization of different species of prawns renders it possible to understand the extent to which prawn stocks differ genetically along their spatial range of distribution. In other words delineation of stocks, which is one of the most essential parameters necessary for effective management of a fishery, is made easier. The results obtained in the present study are expected to help in a big way in solving some of the problems envisaged.

CHAPTER II

MATERIALS AND METHODS

Collection of specimen

The specimens for extraction of organs and materials for study were collected live from the catches. Different species of prawns for analysis were collected from different centres as shown in Figure 1. For instance Penaeus indicus was collected from four different centres as shown in Table 1. In Cochin backwaters the white prawn was taken from cast net and chinese dip net catches. Live specimens were also collected from prawn culture laboratory at Narakkal. In addition collection of Penaeus indicus and Parapenaeopsis stylifera were made by operations of trawl nets from CMFRI Research Vessel Cadalmin. (Plate 13,14)

In Tuticorin material was collected from trawl net operated by CMFRI Research Vessel Cadalmin and in Waltair from the nets operated by research vessel there. Collection in Madras was made from the catches of local catamaran fisherman in Kovalam and from Pentakota fish landing centre at Puri.

Table 1: Distribution and collection sites of Penaeid prawn species studied.

| Species | Common Name | Site of Collection | Distribution |
|--|--------------------|--|---|
| <u>Penaeus indicus</u> H. Milne Edwards, 1837 | Indian White Prawn | Cochin Tuticorin, Madras, Waltair | Kenya, Persian Gulf, Indo-Pacific, E. and S. Africa to S. China, New Guinea and Australia. |
| <u>Penaeus japonicus</u> Bate, 1888 | Kurma prawn | Madras | Indo-west Pacific from the Red sea, E. and S.E. Africa to Korea, Japan and Malaya Archipelago, In India Bombay and Madras coasts. |
| <u>Penaeus canaliculatus</u> (Oliver, 1811) | Witch prawn | Madras | Indo-west Pacific, Madras, Cochin and Bombay in India. |
| <u>Penaeus latisulcatus</u> Kishinouye, 1896 | King prawn | Madras | Mosambique, Southern Redsea, Somalia, Gulf of Aden and the Persian Gulf, Japan. South West coast of India. |
| <u>Penaeus merguensis</u> De Man, 1888 | Banana Prawn | Puri | Indo-west Pacific from Persian Gulf to Thailand, Malaya, Hong Kong, Philippines, India Karwar of W. India, Puri, and Paradeep. |
| <u>Penaeus penicillatus</u> Alcock, 1905 | Red tail prawn | Puri | Indo-west Pacific from Pakistan to Taiwan, Malaya and Indonesia Maharashtra coast. |

| Species | Common Name | Site of Collection | Distribution |
|---|----------------|--------------------|--|
| <u>Metapenaeus brevicornis</u> (H. Milne Edwards, 1837) | Yellow prawn | Bombay | Pakistan, N.W. India, Ganges, Delta of W. Bengal, Bangladesh, W. coast of Thailand, Malaya and Indonesia. |
| <u>Metapenaeus affinis</u> (H. Milne Edwards, 1837) | Jinga prawn | Bombay | Indo-west Pacific, Arabian Sea to the Malaya, Archipelago and Hongkong. In India along the West coast of India and the Southern part of east coast |
| <u>Metapenaeus kutchensis</u> George, George & Rao, 1963 | Ginger prawn | Bombay | Gulf of Kutch and N.W. India |
| <u>Metapenaeus monoceros</u> (Fabricius, 1798) | Speckled prawn | Bombay | Indo-west Pacific, E. and S.E. Africa, Red Sea to Bay of Bengal. In India most of the coast. |
| <u>Parapenaeopsis stylifera</u> (H. Milne Edwards, 1837) | Kiddi Prawn | Cochin, Bombay | Indo-west Pacific, Persian Gulf, All along the coast line of India. |
| <u>Parapenaeopsis hardwickii</u> (Miers, 1878) | Spear prawn | Bombay | Indo-west Pacific, In India Bombay and Godavary estuary. |
| <u>Parapenaeopsis sculptilis</u> (Heller, 1862) | Rainbow prawn | Bombay | Indo-west Pacific from Pakistan to Malaya. In India Bombay and Ganges delta |

Fig. 1

MAP SHOWING COLLECTION SITES

- Penaeus indicus
- Penaeus japonicus
- Penaeus canaliculatus
- Penaeus latisulcatus
- △ Penaeus merguiensis
- ▲ Penaeus penicillatus
- ⊙ Parapenaeopsis stylifera
- ⊗ Parapenaeopsis sculptilis
- ◐ Parapenaeopsis hardwickii
- ▤ Metapenaeus brevicornis
- ⊠ Metapenaeus affinis
- × Metapenaeus kutchensis
- ⬡ Metapenaeus monoceros

Tuticorin

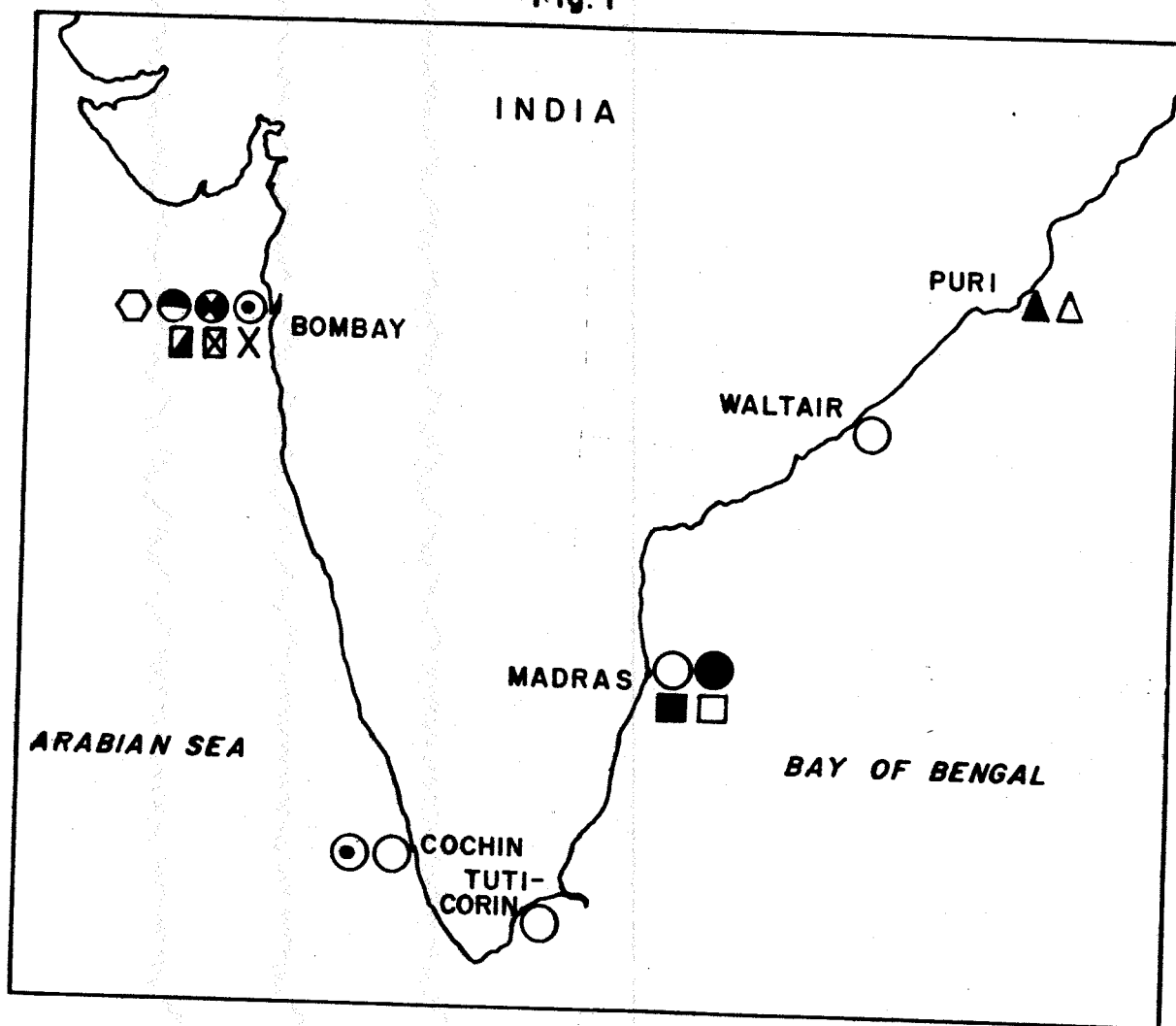
Cochin

Madras

Waltair

Puri

Fig. 1



Sample preparation

All enzymatic proteins were analysed in 3 different tissues. Viz. eye, hepatopancreas and muscles tissues. Prawns of intermoult stages with immature gonad conditions were collected. For larval stages whole animal homogenized tissue extract was taken. Tissues taken from freshly sacrificed prawns were dissected in ice cold condition. Tissue was washed with precooled distilled water and the water content was removed by wiping it with blotting paper. Definite quantity of tissue was measured and homogenized with ice cooled distilled water in mechanical homogenizer at 80 rpm. inside ice box. Then it was centrifuged for 10,000 rpm at 4°C for 20 mts. The supernatant was taken and it was freezeed for further use in electrophoresis. The quantity of protein used as the sample loaded for electrophoresis was determined by Lowry's method (1951).

Electrophoresis:

Simplified procedure of zone electrophoretic separation of serum protein is found to be the best method for separation of isoenzymes. The ability of a protein molecule to migrate in an electric field depends on its net electric charge and size. According to the pH of buffers protein can be made to travel towards either electrode. In alkaline

solution most protein are negatively charged and travel towards anode. Polyacrylamide gel medium was used here for electrophoresis.

Separating Gel preparation:

Polyacrylamide gel is stable, non-reactive with sample, inert and the pore size can be adjusted by addition of various concentrations of bisacrylamide. Different percentage of the polyacrylamide gel was prepared as given in the polyacrylamide gel electrophoretic method of Laemmli (1970).

30% of Acrylamide stock was prepared as follows:-
30 gm of acrylamide and 0.8 gm of bisacrylamide was dissolved in double distilled water and made upto 100 ml. This was filtered by multipore filterpaper No.42. For preparing 10% concentration of acrylamide from the 30% acrylamide stock, following calculation were made

$$\begin{aligned} V_1 N_1 &= V_2 N_2 \\ V_2 &= 30 \text{ ml} \\ N_2 &= 10\% \\ N_1 &= 30\% \end{aligned}$$

$$\text{So } V_1 = \frac{30 \times 10}{30} = 10 \text{ ml.}$$

Likewise 5%, 7.5%, 10%, 12.5% and 15% concentration of acrylamide can be prepared from the above stock solution and bisacrylamide concentration can also be changed.

Volume of separating gel buffer was estimated as given below.

Separation gel buffer pH (8.9)

36.6 gm of Tris (Hydroxymethyl) was dissolved in double distilled water. The pH was adjusted with IN HCl and the final volume was made upto 100 ml with distilled water. The concentration is 3 molar. But for using 0.75 molar is necessary. So the following formula is used.

$$V_1 N_1 = V_2 N_2$$

$$V_1 \times 3 = 30 \times .75$$

$$V_1 = \frac{30 \times .75}{3} = 7.5$$

$$V_1 = 7.5 \text{ ml.}$$

So volume of separating gel buffer is 7.5 ml. for 30 ml. solutions. The amount of acrylamide and the total volume of distilled water will change according to different percentage of acrylamide. With this 20 μ l of Tetramethylenediamine (TEMED) is usually added to serve as a catalyst of gel formation because it exist as a free radical. Oxygen inhibits polymerization of gels because it eliminates free radicals. Hence the solution is degased and gel is formed in air tight chambers. 90 μ l

of 10% of Ammonium persulphate was added to the above solution to enhance the polymerization of the gel. This volume has to be subtracted from the total 30 ml. The remaining is made up with double distilled water.

For preparing 7.5% of acrylamide the following volume of solution were taken

| | |
|--------------------------|-------------|
| Acrylamide 30% stock | 7.5 ml |
| Separating gel buffer | 7.5 ml |
| Water | 14.89 ml |
| TEMED | 20 μ l |
| 10% Ammonium persulphate | 90 μ l |
| Total | <hr/> 30 ml |

Spacer gel preparation:

Large pore buffer, acrylamide solution and riboflavin were used to prepare this.

Large pore buffer.

Dissolve 5.98 gm of Tris (Hydroxymethyl) in double distilled water. Add 0.46 ml of TEMED and adjust pH to 6.7 with 1N HCl and make it upto 100 ml with distilled water.

Acrylamide solution:

Dissolve 10gm of acrylamide and 2.5 gm of methylene bisacrylamide in 100 ml of distilled water to get 3% concentration of spacer gel. 0.04% of riboflavin was prepared and used for polymerization of the gel in the presence of UV light to form free radicals. The above solution were mixed in 1:2:1 and the spacer gel is prepared. Main function of this gel is to arrange the different molecules depending on its size.

The acrylamide stock solutions, separating gel buffer and spacer gel buffer are prepared and stored in amber colour bottles in a refrigerator. Ammonium persulphate is prepared fresh every day before use.

Gel casting:

Gels are moulded in the form of gel rods. Polyacrylamide gels are made in gel tubes of desired size. In continuous gel electrophoresis gel pore size and buffer is one kind and in discontinuous gel electrophoresis pore size of the gel is of two kinds. In Disc gel electrophoresis some times two types of gels viz. separating gel and spacer gel are used for general proteins and separating gel only is used for enzymes. Glass tubes both end opened and having

uniform diameter (.5cm) and length of 7.5 cm was selected. They are placed in a suitable stand in vertical position. The gel tubes were placed in the gel stand and one end of the tube sealed by rubber cork.

Separating gel solution is prepared and it was poured in each gel tube from the sides of the tube with a filler upto the first scratch mark. Care was taken to avoid bubble formation while pouring the solution. After this one drop of water was added from the sides of the gel tube to avoid miniscus formation. When the polymerization is over remove the upper layer of water with the blotting paper completely and carefully. Spacer gel solution was prepared as mentioned above for general proteins. This solution was poured as before upto the second mark. Now also a drop of water is added from the sides to avoid miniscus and allow it for polymerization. The water layer is discarded after polymerization. The gels were placed inside the refrigerator for half an hour before use. Then the sample for analysis in particular quantity was taken by a microliter syringe. This is mixed with 10 μ l of 0.1% aqueous bromophenol blue and 40 μ l of 40% sucrose. After the addition of all the above, the sample form the third layer in the gel. Remove the gel tubes from the cork and

place one drop of electrode buffer in the left out portion of tube to avoid bubble formation. Then the gel tubes are inserted into the grommets of the upper buffer tank.

Electrode buffer is poured in the upper tank and lower tanks from the sides of the tank after proper dilution with the distilled water.

Then the electrical connections were made between the disc electrophoresis which is placed inside the BOD incubator at 4°C and the Electrophoretic power pack. Power pack is adjusted in such a way to pass current of 4mA per gel tube or 200-240 V for general protein. This passage of current is adjusted in a different way for different enzymes. According to the charge and size of the protein it will move in the gel. After the bromophenol blue comes to the lower edge of the gel tubes the supply of current is terminated. Buffer is poured out. Gel tubes are removed from the grommet. Distance travelled by the bromophenol blue was found out. The gels in the tubes are removed by injecting water in between the gel and the tube with the help of a syringe. Staining was carried out for different proteins as given in the histochemical staining of proteins. Mobility of each fraction was measured from the point of application. Relative mobility was calculated. The gel was preserved in 7% acetic acid and photographed.

The enzyme which is separated by electrophoresis can be detected by stripping and incubating the test tube at 37°C. With the gel a solution containing substrate for each enzyme, cofactor NAD, electron acceptor PMS, electron indicator NBT one buffer to maintain pH were added.

Conditions were kept constant for all the studies. Same apparatus and power pack were used throughout this study to avoid any experimental error. The set up used for this purpose is shown in photograph (Plate 1).

Vertical gel electrophoresis

Polyacrylamide vertical slab system was employed for the separation of general protein patterns of Panaeus indicus. 12 cm x 12 cm length slabs having 1 mm thickness was used for this purpose. Spacers were kept in the 2 extreme ends of the slab in the parallel manner leaving 0.5 cm space in the ends. The slab was placed inside the lower buffer chamber and kept in position by the clips. Then the three sides of the slab were sealed off with agar gel. The preparation of acrylamide solution and other buffers are same as already explained in disc electrophoresis. Solution is poured with a filler from one side of the slab. The comb is placed in the anterior end of the

slab to form slots for loading the sample. After the polymerization the comb is removed and the sample was applied on the slots by microlitre syringe. Both the upper and lower tanks were filled with the buffer. This set up was kept in BOD at 4°C. The electrical connections were made with the power pack. For each slot 4 mA current was applied. When the bromophenol marker reaches the end the passage of electricity was terminated. The mobility distance of the bromophenol blue was measured. The gel is stained for general protein. The set up used for doing slab gel electrophoresis is shown in photograph. (Plate 2) This method was employed for various other enzymes but the separation and resolution was not good like what is resolved in disc electrophoresis. So disc gel electrophoresis method was employed for enzymes and general protein separation (Plate 3) of P. indicus was carried out using slab gel electrophoresis method.

Histochemical staining of gel:

After the electrophoresis the gels were incubated in the staining solution for the appearance of characteristic protein bands. Different staining components used for specific enzymes are given below:-

Acid phosphatase (Siciliano & Shaw, 1976)

Combine the following in a flask/Sodium alpha naphthylacid phosphate 50mg/Fast Garnet Blue Salt 50 mg/ water 50 ml/Put the gel in the above solution and incubate at 37°C for 30 minutes. Red bands indicate zones of activity.

Alcohol dehydrogenase (Siciliano & Shaw, 1976).

The following components were mixed and used.

| | |
|------------------------|-------|
| 95% ethanol | 2 ml |
| NAD | 25 ml |
| NBT | 15 mg |
| PMS | 1 mg |
| 0.2 M Tris H cl pH 8.0 | 7 ml |
| Water | 41 ml |

The stain thus prepared was incubated at 37°C after soaking the gel in the stain and keeping it in dark. Dark blue bands are the expression of enzyme on the gel.

Aldehyde oxidase (Redfield and Salini, 1980)

| | |
|--------------|-------|
| Benzaldehyde | 1 ml |
| NAD | 20 mg |
| MTT | 10 mg |

| | |
|----------------------|-------|
| PMS | 2 mg |
| Water | 50 ml |
| Tris-HCl pH 8 buffer | 50 ml |

Dark blue bands appear as soon as the gel is placed inside the stain.

Aldolase (Siciliano & Shaw, 1976)

Following quantity of different chemicals were added and the staining solution of this enzyme was prepared.

| | |
|--|-----------|
| Fructose 1-6 disphosphate tetrasodium salt | 275 mg |
| NAD | 25 mg |
| NBT | 15 mg |
| PMS | 1 mg |
| Sodium arsenate | 75 mg |
| 0.2 M Tris-HCl pH 8.0 | 10 ml |
| Water | 40 ml |
| Glyceraldehyde 3 phosphate dehydrogenase | 100 units |

When the gel is immersed in the staining solution at 37°C dark blue bands appear as zones of enzyme activity.

Alkaline phosphatase (Redfield and Salini, 1980)

| | |
|-----------------------------------|--------|
| α -Naphthyl acid phosphate | 100 mg |
| Fast Garnet GBC Salt | 100 mg |
| Water | 50 ml |
| Tris-HCl, pH 8.5 | 50 ml |

This stain is incubated at 37°C to get red coloured bands of enzyme activity.

Esterase (Redfield and Salini, 1980)

| | |
|------------------------|--------|
| Alpha Naphthyl acetate | 15 mg |
| Fast blue RR salt | 100 mg |
| Water | 50 mg |
| Tris HCl pH 7.0 | 50 ml |

Esterase activity are indicated by dark brown bands after incubating the gel in the stain at 37°C.

α Glycerophosphate dehydrogenase (Siciliano & Shaw, 1976)

| | |
|-------------------------------|-------|
| Sodium alpha Glycerophosphate | 75 mg |
| NAD | 25 mg |
| NBT | 15 mg |
| PMS | 1 mg |
| 0.2 m Tris HCl pH 8.0 | 10 ml |
| Water | 35 ml |

Gels treated with above solution at 37°C will give dark blue bands of enzyme activity.

Lactate dehydrogenase (Siciliano & Shaw, 1976 modified)

| | |
|-----------------|--------|
| Lithium lactate | 100 mg |
| NAD | 25 mg |
| NBT | 15 mg |

| | |
|-----------------------|-------|
| PMS | 1 mg |
| 0.2 M Tris-HCl pH 8.0 | 10 ml |
| Water | 35 ml |

This stain is poured with gel in dark at 37°C to get dark blue bands.

Malate dehydrogenase (Siciliano & Shaw, 1976)

| | |
|-----------------------|-------|
| Malic acid | 10 mg |
| NAD | 25 mg |
| NBT | 15 mg |
| PMS | 1 mg |
| 0.2 M Tris-HCl pH 8.0 | 10 ml |
| Water | 35 ml |

Surface of gels give dark blue bands when the above solution was incubated at 37°C.

Malic enzyme (Siciliano & Shaw, 1976)

| | |
|-----------------------|-------|
| Malic acid | 10 mg |
| NADP | 15 mg |
| NBT | 15 mg |
| PMS | 1 mg |
| Mg Cl ₂ | 50 mg |
| 0.2 M Tris-HCl pH 8.0 | 10 ml |
| Water | 35 ml |

Gels were treated with this stain solution in dark at 37°C to get dark blue bands.

Octanol dehydrogenase (Redfield and Salini, 1980)

| | |
|-----------------|-------|
| Octanol | 3 ml |
| NAD | 20 mg |
| MTT | 10 mg |
| PMS | 2 mg |
| Water | 47 mg |
| Tris-HCl pH 8.5 | 50 ml |

This gel was incubated inside the stain at 37°C to get pink coloured bands to note the presence of enzyme.

6-Phosphogluconate dehydrogenase (Siciliano & Shaw, 1976)

| | |
|---------------------------------------|--------|
| 6-Phosphogluconic acid Trisodium salt | 100 mg |
| NADP | 15 mg |
| NBT | 15 mg |
| PMS | 1 mg |
| Mgcl ₂ | 50 mg |
| 0.2 M Tris HCl pH 8.0 | 10 ml |
| Water | 40 ml |

The gels were incubated at 37°C to get dark blue bands to indicate the presence of enzyme.

Peroxidase (Siciliano & Shaw 1976)

- a. Add 1N HCl to 50 ml of electrode buffer until pH reaches 6.0. Soak gel in this solution for 45 minutes at 4°C and pour off.

b. Pour on solution containing

| | |
|---------------------|-------|
| KI | 1 g |
| Water | 50 ml |
| Glacial acetic acid | 1 ml |

c. Soak gel in above for 60 seconds and wash thoroughly (3 rinses) with distilled water and add the following solution.

| | |
|-------------|-------|
| Water | 50 ml |
| 3% H_2O_2 | 1 ml |

d. Incubate in 37°C until peroxidase appears as dark blue bands on a light blue background.

1-Pyrroline dehydrogenase (Redfield and Salini, 1980)

| | |
|-------------------------|-------|
| Alpha Pyroglutamic acid | 50 mg |
| NAD | 20 mg |
| MTT | 10 mg |
| PMS | 2 mg |
| Water | 47 ml |
| Tris-HCl pH 8.0 | 50 ml |

After adding the stain with the gel it is incubated at 37°C to get blue bands of this enzyme.

Sorbitol dehydrogenase (Redfield and Salini, 1980)

| | |
|------------|-------|
| D-sorbitol | 1 gm |
| NAD | 20 mg |

| | |
|-----------------------|-------|
| MTT | 10 mg |
| PMS | 2 mg |
| Water | 47 ml |
| 0.2 M Tris-HCl pH 8.0 | 50 ml |

Gels were incubated at 37°C to get blue bands of enzyme presence.

Tetrazolium oxidase (Siciliano & Shaw, 1975)

| | |
|-----------------------|--------|
| NADP | 9 mg |
| NBT | 9 mg |
| PMS | 4.5 mg |
| 0.2 M Tris-HCl pH 8.0 | 6 ml |
| Water | 24 ml |

Colourless bands appear after the treatment of the gels with this stain at 37°C.

Standardisation:

Buffers: For genetic variation study particular protein system has to satisfy the following criteria. Bands of activity should be sharp and distinct in order to find the difference in migration rate and the protein has to be located in a tissue which is routinely tested. So a variety of buffers given in different literature have been used for electrophoresis. Buffers resist changes in H^+ and OH^- ion

concentration and maintain constant pH. Each has its own buffering capacity. Buffer system which gives optimum electrophoretic resolution for protein was selected from the following eight buffers.

Buffer system used on different proteins

I. Described by Siciliano and Shaw (1976)

Tris-citrate (TC)

Electrode buffer pH 7.0

Tris-16.35 g

Citric acid (granular monohydrate) - 9.04 g

Water upto 1 L.

Adjust pH upward with 10 N NaOH or downward with Con.

HCl for final pH of 7.0

Gel buffer pH 7.0

Dilute 40 ml of electrode buffer upto 600 ml 4 mA per tube gave good resolution.

II. Described by Siciliano and Shaw (1976)

Tris-Versene-Borate (TVB) pH 8

Electrode buffer

Tris 60.6 g

Boric acid 40.0 g

Na₂ EDTA 2H 20 6.0 g

Water upto 1 L

Adjust pH upward with 10 N NaOH or downward with Com HCl
for final pH 8.0

Gel buffer pH 8.

Dilute 60 ml of electrode buffer upto 600 ml with
water. 4 mA per tube gives good separation.

III. Described by Ferguson and Wallace (1961).

Tris-citric-boric/LiOH (TCBL).

Electrode buffer pH 8.26

LiOH 2.51 g/l

Boric acid 18.54 g/l

Gel buffer pH 8.31

Tris 3.63 g/l

Citric acid 1.05 g/l

Electrode buffer 10 ml/l

Passage of current was restricted to 2 mA per tube.

IV. Described by Richardson (1982).

Stock solution (TM)

Tris 1.211 gm/L

pH adjusted to 7.8 with maleic acid. The solution was
diluted to 1: 10 for gel preparation and used undiluted
for electrode buffer.

Adjust current to 4 mA per tube.

V. Described by Brewer (1970).

Electrode buffer (TME)

| | |
|-------------------|----------|
| Tris | 12.114 g |
| Maleic acid | 11.607 g |
| EDTA Na.2 | 3.7224 g |
| MgCl ₂ | 2.033 g |

The pH is adjusted to 7.6 with 4 N NaOH

Gel buffer:

| | |
|----------------------|-----------|
| Tris | 1.2114 g |
| Maleic acid | 1.1607g |
| EDTA Na ₂ | 0.37224 g |
| Mgcl ₂ | 0.2033 g |

The pH is adjusted to 7.6 with 4 N NaOH.

The gel buffer is a 1:10 dilution of electrode buffer.

Each gel tube was given 2 mA current.

VI. Described by Davis (1964)

Electrode buffer (TG) pH 8.3

| | |
|------------|--------|
| Tris | 6 g |
| Glycine | 28.8 g |
| Water upto | 1 L |

1:10 dilution of this stock solution was used

Gel buffer (TH) pH 8.9

| | |
|--------|--------|
| Tris | 36.6 g |
| 1N HCl | 48 ml |

Water upto 100 ml

VII. Described by Shaw and Prasad (1970).

Stock solution pH 6.8

| | | |
|--|-----------|--------|
| $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ | 27.8 g/L | 255 ml |
| $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ | 53.65 g/L | 245 ml |

Stock solution is undiluted and used as electrode buffer.
65 ml of Electrode buffer was diluted to 1L and used as
gel buffer pH 7.0.

VIII. Described by Brewer (1970).

Electrode buffer pH 7

Sodium citrate 120.581 gm/L

pH is adjusted to 7.0 with 0.41 M citric acid

1:10 dilution was used.

Gel buffer

Histidine 0.7758 g/L

pH is adjusted to 7.0 with 2 N NaOH.

Extraction medium

Tissues were extracted in different solvents to find
out the best solvent which gives good resolution without any
denaturing effect. Definite quantity of tissues were
homogenized in the mechanical homogenizer in ice cold
condition.

Different solvents used for this purpose is given below:

1. Double distilled water.
2. Distilled water and sucrose
3. Tris-HCl mercaptoethanol pH 7.5 (Siciliano & Shaw 1970)
4. Tris-EDTA Na₂ pH 6.8 (Redfield and Salini 1980).

Quantity of sample

To find out optimum concentration of the sample different concentration of sample were tried. For this 50 mg of tissue was homogenized in 1 ml of precooled double distilled water. From this 40 μ l, 50 μ l, 60 μ l and 75 μ l were taken for finding out optimum concentration of sample.

Polyacrylamide gel concentration

As given in gel preparation six different concentration of gels were prepared. viz. 5%, 7.5%, 10%, 12% and 15%. Definite quantity of eye, hepatopancreas and muscle were taken. and the electrophoresis was carried out in different gel concentration. This will be useful in finding out the percentage of acrylamide which gives good resolution and maximum number of bands in general proteins. This acrylamide percentage was tried for different bisacrylamide percentage from 0.8%, 2%, 3%, and 4%. In the above, the percentage which gives good resolution was used for further electrophoretic separation of proteins.

Staining method:

Staining for the general protein of eye, hepatopancreas and muscle was tried with three different stains. Amido black, coomassie Brilliant blue and Kenacid blue. Stains were prepared as given below:

| Stain | Solvent |
|--|--|
| 1. Amido black 0.25 gm | 100 ml of methanol, Water and acetic acid in 5:5:1 ratio |
| 2. Coomassie brilliant blue 0.25 gm | 100 ml of methanol water and acetic acid in 5:5:1 ratio |
| 3. Kenacid blue 0.25 gm | 100 ml of methanol, water and acetic acid in 5:5:1 ratio |
| 4. Kenacid blue 0.25 gm | 100 ml of water |

Storage effect

Effect of storage on different enzymatic proteins like, 1-Pyrroline dehydrogenase, Alcohol dehydrogenase, Aldolase, 6-Phosphogluconate dehydrogenase, Alpha Glycerophosphate dehydrogenase, Malate dehydrogenase and Acid phosphatase were done to find the effect of temperature variation on the activity of tissues. The samples were tried again and again after partial thawing.

Different Parameters analysed

Morphometric analysis: Eleven morphometric dimensions were taken for this work. All the morphometric variables were measured using vernier calipers. Circumference of the abdomen was taken from metric tape. Weights of the specimens were measured by beam balance.

| <u>Code</u> | <u>Variable</u> |
|-------------|--|
| SSL | Length of the sixth abdominal segment along the mid dorsal line. |
| FSL | Length of the first abdominal segment along the mid dorsal line with the prawn extended. |
| PCL | Partial carapace length from the margin of the orbit to the posterior edge of the carapace. |
| CW | Width of the carapace at the point of the last dorsal rostral tooth |
| FLF | Length of the fifth abdominal segment when the prawn is flexed ventrally. |
| SSD | Depth of the abdomen at the mid point of the sixth segment. |
| SAD | Depth of the abdomen at the intersection of the second and the third segments. |
| AAC | Circumference of the abdomen at the intersection of the second and third abdominal segments. |

| | |
|-----|--|
| PAC | Circumference of the abdomen at the intersection of segment five and six |
| TW | Total weight of the prawn specimen. |
| TL | Total length from the tip of the rostrum upto the tip of the telson. |

The above measurements were taken on Penaeus indicus collected from Cochin, Tuticorin, and Madras and Parapenaeopsis stylifera collected from Bombay and Cochin.

Fig 50. Univariate and multivariate analysis was carried out to find out the possible variation within the species.

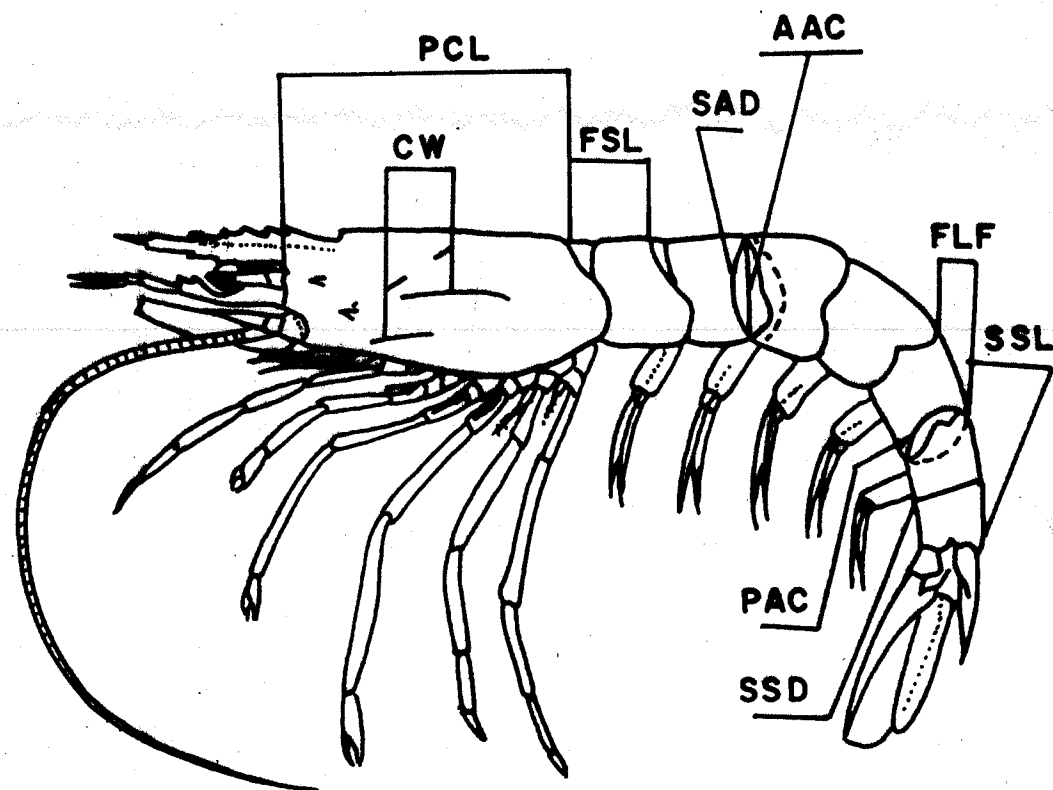
Genetic variation analysis

The enzymes examined for electrophoretic separation were:-

Acid phosphatase (ACPH, E.C.3.1.3.2), Alcohol dehydrogenase (ADH, E.C. 1.1.1.1), Aldehyde oxidase (AO, E.C.1.2.3.1), Aldolase (ALDO, E.C.4.1.2.13), Alkaline phosphatase (ALPH, E.C.3.1.3.1), Esterase (EST, E.C. 3.1.1.1), Alpha Glycero-phosphate dehydrogenase (GPDH, E.C.1.1.1.8), Lactate dehydrogenase (LDH, E.C. 1.1.1.27), Malate dehydrogenase (MDH, E.C. 1.1.1.37), Malic enzyme (ME, E.C. 1.1.1.40), Octanol dehydrogenase (ODH, E.C. 1.1.1.73), 6-Phosphogluconate dehydrogenase (6-PGDH, E.C. 1.1.1.44), 1-Pyrroline dehydrogenase (1-PYDH, E.C. 1.5.1.12), Peroxidase (E.C.1.11.1.7),

Fig. 50. Metric variables used in the multivariate analysis for Penaeus indicus and Parapenaeopsis stylifera.

Fig.50



Sorbitol dehydrogenase (SDH, E.C. 1.1.1.14), and Tetrazolium oxidase (TO, E.C. 1.15.1.1).

Genetic expression and variation in different tissues were carried out on P. indicus and P. stylifera collected from the above localities. Electrophoretic separation of above mentioned enzymes were analysed in tissues like eyes, hepatopancreas and muscles.

Ontogeny:

Different larval stages and juvenile stages as given below have been collected for this study on P. indicus. In Larval stages whole tissue extract was taken and used as sample. But in juveniles the different tissues like eyes, hepatopancreas and muscle were taken and used for electrophoresis. The different proteins analysed were Acid phosphatase, Aldehyde oxidase, Octanol dehydrogenase, Esterase, Alcohol dehydrogenase and Malate dehydrogenase. Different groups tested were: Protozoa, Mysis, Post larvae, Juveniles of 20-30mm, 40-50 mm and 70-80 mm and adults 90-120 mm. The larvae for study were collected from Prawn culture laboratory Narakkal. Protozoa stage was collected for standardization procedure. Whole tissue extract was taken by 15% dilution. All these were done in ice cold condition. Then the extract was used for electrophoresis of different enzymes. Particular

volume required for best resolution was determined by using different quantity of the sample. The particular concentration which gave best resolution was used for further studies on ontogeny. For the juvenile stages and adults particular tissues which gave best resolution was used.

Inter species variation

From Madras Muthukad backwaters three species of closely allied species like Penaeus japonicus, P. canaliculatus and P. latisculcatus were collected. The general protein pattern among these species were found out. Muscle tissue was used for this studies.

Penaeus merquiensis and P. penicillatus was collected from Pentakota fish landing centre at Puri. General protein pattern in muscle tissue was found out. The gels were photographed and scanned in Madurai-Kamaraj University, Madurai.

Allied species of Metapenaeus were collected from Bombay coast. Metapenaeus affinis, M. kutchensis, M. monoceros, M. brevicornis were collected from New Ferry Wharf. Electrophoretic separation of muscle tissue was carried out.

Parapenaeopsis species of prawns were also collected from Bombay. P. hardwickii, P. sculptilis and P. stylifera were collected from season dock. General protein pattern of

muscle was found out for these species. Relative mobility was found out for each band and electrophorograms were prepared. The gels were photographed.

Analysis of electrophoretic data

Each protein fraction separated on the gel during electrophoresis were given numbers from slowest migrating cathodal end towards fastest migrating anodal direction in an increasing order. R_m values were calculated as using the following formula.

$$\text{Relative mobility} = \frac{\text{Distance migrated by the protein fraction} \times 100}{\text{Distance migrated by the marker.}}$$

The frequency of an allele is calculated by $\frac{2H_o + H_e}{2N}$

Where H_o = Number of homozygotes for that allele

H_e = Number of heterozygotes for that allele

N = Number of individuals examined.

Hardy-Weinberg distribution

If a population is in Hardy-Weinberg equilibrium then the frequencies of genotypes will be in the ratio of p^2 , $2pq$ and q^2 for a 2 allele polymorphism where p is the frequency of allele A and q is the frequency of allele B. The difference between observed and expected values of allelic frequency was tested for Chi-Square method.

$$\chi^2 \text{ method} = \frac{(\text{observed frequency} - \text{expected frequency})^2}{\text{Expected frequency}}$$

Nei's coefficient of genetic identity (I) was used

$$I = \frac{x_i y_i}{x_i^2 y_i^2}$$

Where x_i and y_i are the frequencies of the i th allele in populations x and y respectively. The genetic distance (D) is estimated by $D = -\ln I$ which can be obtained from tables of natural logarithms.

Genetic identity and genetic distance between population using Nei's method and Roger's method were carried out using a computer programme developed by Green (1979).

CHAPTER III

STANDARDIZATION OF METHODOLOGY

In the present study different procedures were modified and method which gives better result was used for further analytical work using P. indicus as a test species. For the extraction of enzymes four different homogenizing mediums were used as solvents. Double distilled water showed better results in P. monodon (Prathibha 1984) and in Homarus americanus (Odense and Anand 1978). Since best resolution was obtained using double distilled water it was used to extract protein and enzymes in all the experiments (Table 2).

Staining procedure for general protein was tried with different stains such as coomassie Brilliant Blue, Amidoblack and Kenacid blue. Kenacid blue produced good result. When this was compared with coomassie Brilliant blue Kenacid blue doesn't stain the gels. Whereas coomassie Brilliant blue stains the surface of gel. Kenacid blue was considered to be giving superior staining than the other stains. A mixture of methanol water and acetic acid was used as solvent for dissolving the stain. Here without these solvent the stain Kenacid blue dissolved in distilled water gave equally good results.

Table 2: Effect of different mediums on the resolution of muscle proteins of Penaeus indicus.

| Extracting Medium | Total no. of bands | Resolution | Training |
|--|--------------------|------------|----------|
| 1. Double distilled water | 13 | Good | Nil |
| 2. Double distilled water and sucrose | 10 | Poor | Heavy |
| 3. Tris-Hcl, Mercapto-ethanol pH 7.5 (Siciliano & Shaw 1970) | 11 | Fair | Moderate |
| 4. Tris-Edta Na ₂ pH 6.8 (Redfield and Salini, 1980) | 10 | Fair | Moderate |

So Kenacid blue was used for the staining of the general proteins (Table 3).

Quantity required for the optional resolution was determined. Since the quantity of protein content in different tissues vary, different concentration of tissues like eye, hepatopancreas and muscle were tried. For the penaeid prawns Sriraman and Reddy (1977) and Thomas (1981) used 50 mg. and 500 mg per ml. of solvents respectively. Here experiments were carried out, to find the desired quantity of sample which gives good resolution from the sample got by homogenizing 50 mg. of tissue in 1 ml. of double distilled water. From the results it is concluded that 50 μ l for eye and hepatopancreas tissues and 60 μ l for muscle tissue gives good separation without trailing. So these quantity of sample was used in the forth coming experiments also (Table 4).

One more way of improving the resolution is by varying the concentration of separating gel. The proteins of high molecular weight are separated using gels of larger pore size and the smaller proteins are separated in a better way using gels of smaller pore size. The upper range of Monomer concentration for acrylamide gel has to be adjusted to get a suitable gel concentration (Smith 1968). Most of the

Table 3: Muscle myogen protein pattern of Penaeus indicus in different stains

| Staining Mixture | Staining Intensity | | | |
|--|--------------------|--------|-------|-----------------------|
| | Dark | Medium | Light | Total number of bands |
| | | | | Background colour |
| 1. Amido black in 100 ml of methanol, water and acetic acid in 5:5:1 ratio | 5 | 6 | | 11 Bluish black |
| 2. 0.25 m of Coomassie brilliant blue GR-250 | 5 | 7 | | 12 Pale blue |
| 3. 0.25 gm of kenaeid blue in 100 ml of methanol, water and acetic acid in 5:5:1 ratio | 5 | 8 | | 13 Clear |
| 4. 0.25 gm of kenaeid blue in 100 ml of water | 5 | 8 | | 13 Clear |

Table 4: General Protein patterns of Penaeus indicus using different quantities of Eye, hepatopancreas and muscle tissues.

7.5%

| Sample Volume | Protein μgm content | No. of bands | Resolution | Trailing |
|------------------------------|---|---------------------|-------------------|-----------------|
| <u>Eye</u> | | | | |
| 40 μ l | 264 | 10 | Good | Nil |
| 50 μ l | 330 | 11 | Good | Nil |
| 60 μ l | 396 | 11 | Fair | Moderate |
| 75 μ l | 495 | 11 | Poor | Moderate |
| <u>Hepatopancreas</u> | | | | |
| 40 μ l | 103 | 9 | Good | Nil |
| 50 μ l | 129 | 11 | Good | Nil |
| 60 μ l | 155 | 11 | Fair | Moderate |
| 75 μ l | 194 | 11 | Fair | Moderate |
| <u>Muscle</u> | | | | |
| 40 μ l | 220 | 11 | Good | Nil |
| 50 μ l | 275 | 12 | Good | Nil |
| 60 μ l | 330 | 13 | Good | Nil |
| 75 μ l | 413 | 13 | Fair | Moderate |

investigation on prawns were carried out using 7% acrylamide (Sriraman and Reddy 1977, Thomas, 1981) whereas 10% acrylamide (Prathibha 1984) concentration was found to produce best resolution for proteins extracted from eye, hepatopancreas muscle and serum of P. monodon. Likewise here also 10% acrylamide with 0.8% bisacrylamide concentration gives best resolution than the other gel concentration which were experimentally tried and resulted in poor resolution (Table 5).

Storage effect:

Effect of storage on different enzymatic proteins like 1-Pyrroline dehydrogenase, Alcohol dehydrogenase, Aldolase, 6-Phosphogluconate dehydrogenase, Alpha Glycerophosphate dehydrogenase, Malate dehydrogenase and Acid phosphatase was tried. This was analysed for 3 days. Studies revealed that except Aldolase, 6-Phosphogluconate dehydrogenase, all the other enzymes didn't show any variation in their intensity. This proves that the enzymatic activity was not influenced by the storage. All the enzymes were tested with eight different buffers to find out their resolution. Acid phosphatase resolved well in Tris citrate buffer pH 7 (Table 6).

Table 5: Protein separation using different ratios of Acrylamide and Bisacrylamide.

| Tissues | Concentration of Acrylamide and Bisacrylamide % with no.of bands | | | | | | | | |
|-----------------------|--|---------|---------|----------|--------|--------|-------|------|------|
| | 5,0.8 | 7.5,0.8 | 10,0.8, | 12.5,0.8 | 15,0.8 | 10,0.8 | 10, 2 | 10,3 | 10,4 |
| Eye | 3 | 10 | 14 | 7 | 5 | 14 | 11 | 5 | 6 |
| Hepatopancreas | 6 | 12 | 17 | 12 | 11 | 17 | 15 | 15 | 7 |
| Muscle | 5 | 10 | 20 | 10 | 9 | 20 | 18 | 17 | 15 |

Table 6: Phosphatase (ACPH 3.1.3.2) resolution of different tissues of Penaeus indicus in different buffers

| Electro-phoretic system | Tis-sues | No.of bands | Intensity of band | | | | | Distin-ction | Separ-ation |
|-------------------------|----------|-------------|-------------------|----|------|------|----|--------------|-------------|
| | | | 1 | 2 | 3 | 4 | 5 | | |
| TC I | E | 2 | xx | xx | 0 | 0 | 0 | + | + |
| | H | 4 | xx | xx | 0 | x | xx | + | + |
| | M | 1 | 0 | 0 | xx | 0 | 0 | + | + |
| TCB III | E | - | 0 | 0 | 0 | 0 | 0 | - | - |
| | H | 2 | 0 | 0 | xx | xxx | 0 | - | - |
| | M | - | 0 | 0 | 0 | 0 | 0 | - | - |
| TM IV | E | - | 0 | 0 | 0 | 0 | 0 | - | - |
| | H | 2 | 0 | 0 | xxxx | 0 | xx | - | - |
| | M | - | 0 | 0 | 0 | 0 | 0 | - | - |
| TME V | E | 2 | 0 | 0 | xx | 0 | x | - | - |
| | H | 2 | 0 | 0 | xxx | xx | 0 | - | - |
| | M | 1 | 0 | 0 | 0 | x | 0 | - | - |
| PH VII | E | 1 | 0 | 0 | 0 | xx | 0 | - | - |
| | H | 4 | 0 | x | xx | xxxx | xx | - | - |
| | M | 1 | 0 | 0 | 0 | xxx | 0 | - | - |
| HSC VIII | E | 2 | 0 | 0 | 0 | xx | xx | - | - |
| | H | 2 | 0 | 0 | 0 | xx | xx | - | - |
| | M | 2 | 0 | 0 | xx | xxx | 0 | - | - |

No resolution in TG VI and TVB II

Distinction : Bands clear without trailing +
Bands diffused with trailing -

Separation : good clear and resolved well
bands with sharpened ends +
Poor -

Band intensities:

| | | | |
|------------------|------|------|---|
| Dark | xxxx | I | - Tris citrate pH 7 (TC) |
| Medium | xxx | II | - Tris-Versene-Borate pH 8(TVB) |
| Light | xx | III | - Tris-citric-Boric-LiOH pH 8,31,8.26(TC) |
| Faint | x | IV | - Tris Maleic acid pH 7.8 (TM) |
| No activity | 0 | V | - Tris maleic acid Edta pH 7.6 (TME) |
| | | VI | - Tris Glycine pH 8.3 (TG) |
| E - Eye | | VII | - Phosphate buffer pH 7(pH) |
| H - Hepatopaneas | | VIII | - Histidine pH 7 Sodium citrate pH 7(HSC) |
| M - Muscle | | | |

Alcohol dehydrogenase enzyme showed good resolution in Tris versene Borate buffer pH 8 (Table 7).

Table 8 showed that Aldehyde oxidase is giving good separation in Tris glycine buffer pH 8.3. Esterase enzyme gave good resolution when tested with Histidine pH 7 and Sodium citrate pH 7 buffers (Table 9). Alpha glycerophosphate dehydrogenase enzyme resolved well in Tris versene Borate.

pH 8 (Table 10). Table 11 showed lactate dehydrogenase to resolve in Tris citric acid (pH 8.31). Lithium Hydroxide buffer (pH 8.26). From the table No.12 it is learnt that malate dehydrogenase resolved well in Tris glycine buffer pH 8.3. Malic enzyme resolved well in Tris-maleic acid Edta buffer pH. 7.6 (Table 13), Octanol dehydrogenase resolved well in Tris Maleic acid buffer (Table 14).

6-Phosphogluconate dehydrogenase showed good resolution in Tris versene borate buffer pH 8 (Table 15).

1-Pyrroline dehydrogenase buffer expressed good resolution in Tris versene Borate pH .8 (Table 16). Table 17 showed Tetrazolium oxidase to resolve well in Tris versene Borate buffer pH 8. Peroxidase showed good resolution in Histidine pH 7 sodium citrate buffer pH.7 since

Table 7: Alcohol Dehydrogenase (ADH 1.1.1.1) resolution of different tissues of Penaeus indicus in different buffers

| Electro-phoretic system | Tis-sue | No. of bands | Intensity of bands | | | | Distin- tion | Separ- ation |
|-------------------------|---------|--------------|--------------------|----|----|----|--------------|--------------|
| | | | 1 | 2 | 3 | 4 | | |
| TC I | E | 2 | 0 | x | x | 0 | - | - |
| | H | 2 | x | xx | 0 | 0 | - | - |
| | M | 1 | 0 | xx | 0 | 0 | - | - |
| TVB II | E | 2 | 0 | xx | xx | 0 | + | + |
| | H | 2 | x | xx | 0 | 0 | + | + |
| | M | 1 | 0 | xx | 0 | 0 | + | + |
| TCBL III | E | 1 | 0 | 0 | xx | 0 | - | - |
| | H | 2 | xx | xx | 0 | 0 | - | - |
| | M | 1 | xx | 0 | 0 | 0 | - | - |
| TM IV | E | 2 | x | 0 | xx | 0 | - | - |
| | H | 2 | x | 0 | x | 0 | - | - |
| | M | - | 0 | 0 | 0 | 0 | - | - |
| TME V | E | 1 | 0 | 0 | xx | 0 | - | - |
| | H | 2 | xxx | 0 | 0 | xx | - | - |
| | M | 2 | xxx | 0 | x | 0 | - | - |
| pH VII | E | 2 | 0 | 0 | x | xx | - | - |
| | H | 2 | xxx | 0 | 0 | xx | - | - |
| | M | 2 | 0 | x | 0 | x | - | - |
| HSC VIII | E | 2 | 0 | 0 | xx | xx | - | - |
| | H | 2 | 0 | 0 | x | x | - | - |
| | M | 2 | xxx | xx | 0 | 0 | - | - |

No resolution in TG VI

Table 8: Aldehyde Oxidase (AO 1.2.3.1) resolution of different tissues of Penaeus indicus in different buffer.

| Electro-phoretic system | Tis-sue | No. of bands | Intensity of bands | | | Distin-ction | Separa-tion |
|-------------------------|---------|--------------|--------------------|------|----|--------------|-------------|
| | | | 1 | 2 | 3 | | |
| TC I | E | - | 0 | 0 | 0 | - | - |
| | H | 2 | xxxx | 0 | xx | - | - |
| | M | 1 | 0 | x | 0 | - | - |
| TVB II | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xx | 0 | - | - |
| | M | - | 0 | 0 | 0 | - | - |
| TCBL III | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xxx | 0 | - | - |
| | M | - | 0 | 0 | 0 | - | - |
| TM IV | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xxx | 0 | - | - |
| | M | - | 0 | 0 | 0 | - | - |
| TME V | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xxxx | 0 | - | - |
| | M | - | 0 | 0 | 0 | - | - |
| TG VI | E | - | 0 | 0 | 0 | - | - |
| | H | 2 | xx | xx | 0 | + | + |
| | M | 1 | 0 | 0 | xx | + | + |
| pH VII | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xx | 0 | - | - |
| | M | - | 0 | 0 | 0 | - | - |
| HSC VIII | E | - | 0 | 0 | 0 | - | - |
| | H | 2 | 0 | xxx | x | - | - |
| | M | - | 0 | 0 | 0 | - | - |

Table 9: Esterase (EST, E.C. 3.1.1.1) resolution of different tissues of Penaeus indicus in different buffers.

| Electro-phoretic system | Tiss-ues | No.of bands | Intensity of bands | | | | Distin-ction | Separa-tion |
|-------------------------|----------|-------------|--------------------|----|----|-----|--------------|-------------|
| | | | 1 | 2 | 3 | 4 | | |
| TC I | E | 2 | xx | 0 | 0 | xxx | - | - |
| | H | 2 | 0 | xx | - | xxx | - | - |
| | M | 2 | xx | 0 | 0 | xx | - | - |
| TVB II | E | 2 | 0 | 0 | xx | xx | - | - |
| | H | 2 | 0 | x | 0 | 0 | - | - |
| | M | 1 | 0 | 0 | 0 | x | - | - |
| TCBL III | E | 1 | 0 | 0 | xx | 0 | - | - |
| | H | 2 | x | 0 | xx | 0 | - | - |
| | M | 1 | xx | 0 | 0 | 0 | - | - |
| TM IV | E | 1 | 0 | 0 | xx | 0 | - | - |
| | H | 2 | x | 0 | x | 0 | - | - |
| | M | - | 0 | 0 | 0 | 0 | - | - |
| TME V | E | 1 | 0 | 0 | 0 | xx | - | - |
| | H | 2 | xxx | 0 | 0 | xx | - | - |
| | M | 2 | xxx | 0 | 0 | x | - | - |
| pH VII | E | 2 | 0 | 0 | x | xx | - | - |
| | H | 2 | xxx | 0 | 0 | xx | - | - |
| | M | 1 | 0 | 0 | 0 | x | - | - |
| HSC VIII | E | 1 | 0 | x | 0 | 0 | + | + |
| | H | 2 | xx | 0 | xx | 0 | + | + |
| | M | 3 | xx | 0 | xx | x | + | + |

**Table 10: Glycerophosphate dehydrogenase (Gpdh 1.1.1.8)
resolution of different tissues of Panaeus
indicus in different buffers.**

| Electro- photetic system | Tissues | No.of bands | Intensity of bands | | Distin- ction | Separa- tion |
|--------------------------------|---------|----------------|--------------------|---|------------------|-----------------|
| | | | 1 | 2 | | |
| TVB | E | - | 0 | 0 | - | - |
| | H | 1 | xx | 0 | + | + |
| | M | - | 0 | 0 | - | - |
| TCBL | E | - | 0 | 0 | - | - |
| | H | 1 | x | 0 | - | - |
| | M | - | 0 | 0 | - | - |

No activity in TC I, TM IV, TME V, TG VI, pH VII, HSC VIII.

Table 11: Lactate dehydrogenase (LDH 1.1.1.27) resolution of different tissues of Penaeus indicus in different buffers.

| Electrophoretic system | Tissues | No. of bands | Intensity of bands | | Distinction | Separation |
|------------------------|---------|--------------|--------------------|------|-------------|------------|
| | | | 1 | 2 | | |
| TC I | E | 1 | x | 0 | - | - |
| | H | - | 0 | 0 | - | - |
| | M | 2 | xx | xx | - | - |
| TVB II | E | 1 | 0 | xx | - | - |
| | H | 1 | xx | 0 | - | - |
| | M | 1 | 0 | xxxx | - | - |
| TCBL III | E | 1 | 0 | x | + | + |
| | H | 1 | x | 0 | + | + |
| | M | 2 | x | xx | + | + |
| TME V | E | 1 | 0 | xx | - | - |
| | H | 1 | xx | 0 | - | - |
| | M | 1 | 0 | xx | 0 | 0 |
| pH VIII | E | 1 | x | 0 | - | - |
| | H | - | 0 | 0 | - | - |
| | M | 1 | x | 0 | - | - |

No resolution in TM IV, TG VI, HSC VIII

Table 12: Malate dehydrogenase (MDH 1.1.1.37) resolution of different tissues of *Penaeus indicus* in different buffers.

| Electro-phoretic system | Tissue | No. of bands | Intensity of bands | | | Distinction | Separation |
|-------------------------|--------|--------------|--------------------|-----|----|-------------|------------|
| | | | 1 | 2 | 3 | | |
| TVB II | E | 1 | x | 0 | 0 | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 2 | 0 | xx | x | - | - |
| TCBL III | E | 1 | 0 | 0 | x | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 2 | xx | x | 0 | - | - |
| TM IV | E | 1 | 0 | 0 | xx | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 1 | x | 0 | 0 | - | - |
| TME V | E | 1 | x | 0 | 0 | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 2 | 0 | xxx | x | - | - |
| TG VI | E | 1 | xx | 0 | 0 | + | + |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 2 | 0 | x | x | + | + |
| pH VII | E | 1 | x | 0 | 0 | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 2 | x | xx | 0 | - | - |
| HSC VIII | E | 1 | x | 0 | 0 | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 2 | 0 | xxx | x | - | - |

No resolution in TC 1.

Table 13: Malic enzyme (ME 1.1.1.40) resolution of different tissues of Penaeus indicus in different buffers.

| Electrophoretic system | Tiss- ues | No. of hands | Intensity of bands | | Distin- ction | Separa- tion |
|------------------------|--------------|-----------------|--------------------|----|------------------|-----------------|
| | | | 1 | 2 | | |
| TC I | E | 1 | 0 | x | - | - |
| | H | - | 0 | x | - | - |
| | M | 2 | x | x | - | - |
| TVB II | E | 1 | 0 | x | - | - |
| | H | - | 0 | 0 | - | - |
| | M | 1 | 0 | xx | - | - |
| TCBL III | E | 1 | 0 | x | - | - |
| | H | - | 0 | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| TM IV | E | 1 | 0 | xx | - | - |
| | H | - | 0 | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| TME V | E | 1 | xx | 0 | + | + |
| | H | - | 0 | 0 | + | + |
| | M | 1 | 0 | x | + | + |
| TG VI | E | 1 | 0 | xx | - | - |
| | H | 1 | x | 0 | - | - |
| | M | 1 | x | 0 | - | - |
| pH VII | E | 1 | 0 | x | - | - |
| | H | - | 0 | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| HSC VIII | E | 2 | x | xx | - | - |
| | H | 1 | 0 | x | - | - |
| | M | - | 0 | 0 | - | - |

Table 14: Octanol dehydrogenase (ODH 1.1.1.73) Resolution of different tissues of Penaeus indicus in different buffers.

| Electro-phoretic system | Tissue | No. of bands | Intensity of bands | | | Distinction | Separation |
|-------------------------|--------|--------------|--------------------|----|----|-------------|------------|
| | | | 1 | 2 | 3 | | |
| TVB II | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | xx | 0 | 0 | - | - |
| | M | - | 0 | 0 | 0 | - | - |
| TCB ₂ III | E | 1 | x | 0 | 0 | - | - |
| | H | 1 | x | 0 | 0 | - | - |
| | M | 1 | xx | 0 | 0 | - | - |
| TM IV | E | 2 | x | x | 0 | - | - |
| | H | 1 | 0 | 0 | x | - | - |
| | M | 2 | x | x | 0 | - | - |
| TME V | E | 1 | 0 | xx | 0 | + | + |
| | H | 1 | xx | 0 | 0 | + | + |
| | M | 1 | 0 | 0 | xx | + | + |
| TG VI | E | 1 | 0 | 0 | x | - | - |
| | H | 1 | x | 0 | 0 | - | - |
| | M | 1 | 0 | x | 0 | - | - |

No resolution in TC I, HSC VIII and pH VII

Table 15: 6-Phosphogluconate dehydrogenase (6 PGDH 1.1.1.44)
Resolution of different tissues of Penaeus indicus in
different buffers.

| Electro- phoretic system | Tiss- ues | No. of bands | Intensity of bands | | Distin- ction | Separa- tion |
|--------------------------------|--------------|-----------------|--------------------|----|------------------|-----------------|
| | | | 1 | 2 | | |
| TC I | E | - | 0 | 0 | - | - |
| | H | 1 | xx | 0 | - | - |
| | M | 1 | 0 | x | - | - |
| TVB II | E | 2 | x | x | + | + |
| | H | 1 | 0 | xx | + | + |
| | M | 1 | 0 | x | + | + |
| TCBL III | E | - | 0 | 0 | - | - |
| | H | 1 | xx | 0 | - | - |
| | M | 1 | 0 | x | - | - |
| TM IV | E | - | 0 | 0 | - | - |
| | H | 1 | x | - | - | - |
| | M | - | 0 | 0 | - | - |
| TME V | E | 1 | 0 | x | - | - |
| | H | - | 0 | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| TG VI | E | 1 | xx | 0 | - | - |
| | H | 1 | xx | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| pH VII | E | 1 | 0 | x | - | - |
| | H | - | 0 | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| HSC VIII | E | 1 | 0 | x | - | - |
| | H | 1 | 0 | x | - | - |
| | M | 1 | 0 | x | - | - |

Table 16 : 1-Pyrroline dehydrogenase (PYDH 1.5.1.12)
Resolution of different tissues of Penaeus
indicus in different buffers

| Electro- phoretic system | Tiss- ues | No.of bands | Intensity of bands | | Distin- ction | Separa- tion |
|--------------------------------|--------------|----------------|--------------------|---|------------------|-----------------|
| | | | 1 | 2 | | |
| TC I | H | 1 | x | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| TVB II | H | 1 | 0 | x | + | + |
| | M | 1 | xx | 0 | + | + |
| TCBL III | H | 1 | x | 0 | - | - |
| | M | 1 | x | 0 | - | - |
| TM IV | H | 1 | xx | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| TME V | H | 1 | x | 0 | - | - |
| | M | - | 0 | 0 | - | - |
| pH VII | H | 1 | x | 0 | - | - |
| | M | - | 0 | 0 | - | - |

No resolution in TG VI, HSC VIII

Table 17: Tetrazolium oxidase (TO 1.15.1.1.) resolution of different tissues of Penaeus indicus in different buffers

| Electro-phoretic system | Tiss-ues | No.of bands | Intensity of bands | | | | Distin-ction | Separa-tion |
|-------------------------|----------|-------------|--------------------|---|---|---|--------------|-------------|
| | | | 1 | 2 | 3 | 4 | | |
| TC I | E | 2 | 0 | x | x | 0 | - | - |
| | H | 2 | x | 0 | x | 0 | - | - |
| | M | 1 | 0 | 0 | x | 0 | - | - |
| TVB II | E | 2 | x | x | 0 | 0 | + | + |
| | H | 2 | 0 | 0 | x | x | + | + |
| | M | 1 | 0 | x | 0 | 0 | + | + |
| TCBL III | E | 2 | x | x | 0 | 0 | - | - |
| | H | 1 | 0 | x | 0 | 0 | - | - |
| | M | 1 | 0 | x | 0 | 0 | - | - |
| TG VI | E | 1 | 0 | x | 0 | 0 | - | - |
| | H | 1 | 0 | x | 0 | 0 | - | - |
| | M | - | 0 | 0 | 0 | 0 | - | - |
| HSC VIII | E | 2 | 0 | x | x | 0 | - | - |
| | H | 1 | 0 | x | 0 | 0 | - | - |
| | M | 1 | 0 | x | 0 | 0 | - | - |

No resolution in TM IV, TMS V, pH VII,

the bands disappeared very quickly, it couldn't be used for further studies (Table 18). Sorbitol dehydrogenase expressed good resolution in Tris glycine buffer pH 8.3 (Table 19).

Table 18: Peroxidase (PER 1.11.1.7) resolution of different tissues of P. indicus in different buffers.

| Electro-phoretic system | Tiss-ues | No. of bands | Intensity of bands | | | Distin-ction | Separa-tion |
|-------------------------|----------|--------------|--------------------|----|----|--------------|-------------|
| | | | 1 | 2 | 3 | | |
| TC I | E | 3 | xx | x | x | + | - |
| | H | 1 | 0 | 0 | x | - | - |
| | M | 1 | 0 | 0 | x | - | - |
| TVB II | E | 2 | 0 | x | x | - | - |
| | H | 1 | 0 | 0 | x | - | - |
| | M | 1 | 0 | 0 | x | - | - |
| TCBL III | E | 1 | 0 | 0 | x | - | - |
| | H | 1 | 0 | 0 | x | - | - |
| | M | 1 | 0 | 0 | x | - | - |
| TM IV | E | 2 | 0 | x | x | - | - |
| | H | 1 | 0 | 0 | x | - | - |
| | M | 2 | 0 | x | x | - | - |
| TME V | E | 3 | x | x | x | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 1 | 0 | 0 | x | - | - |
| PH VII | E | 2 | x | x | 0 | - | - |
| | H | 2 | x | x | 0 | - | - |
| | M | 1 | 0 | x | 0 | - | - |
| HSC VIII | E | 3 | x | x | x | + | + |
| | H | 2 | x | 0 | xx | + | + |
| | M | 3 | x | xx | x | + | + |

No resolution in TG VI

Table 19: Sorbitol dehydrogenase (SDH, E.C.1.1.1.14) resolution of different tissues of Penaeus indicus in different buffers.

| Electro-phoretic system | Tissues | No. of bands | Intensity of bands | | | Distinction | Separation |
|-------------------------|---------|--------------|--------------------|----|----|-------------|------------|
| | | | 1 | 2 | 3 | | |
| TC I | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xx | 0 | - | - |
| | M | 1 | 0 | 0 | xx | - | - |
| TVB II | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | xx | 0 | + | + |
| | M | 1 | x | 0 | 0 | - | - |
| TCBL III | E | - | 0 | 0 | 0 | - | - |
| | H | 3 | x | x | xx | + | - |
| | M | 1 | 0 | x | 0 | - | - |
| TM V | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | xx | 0 | 0 | - | - |
| | M | 1 | x | 0 | 0 | - | - |
| TG VI | E | - | 0 | 0 | 0 | - | - |
| | H | 1 | 0 | 0 | xx | + | + |
| | M | 1 | 0 | x | 0 | + | + |
| HSC VIII | E | - | 0 | 0 | 0 | - | - |
| | H | - | 0 | 0 | 0 | - | - |
| | M | 1 | x | 0 | 0 | - | - |

No resolution in pH VII, TM IV.

CHAPTER IV
INTERSPECIES GENETIC VARIATION

Resume of literature:

Connell (1953 a, b) studied water soluble muscle proteins of fishes using Tiselius technique of electrophoresis for comparative purposes for the first time. Homoir (1955) also studied about fish muscle proteins. Water soluble muscle proteins were analysed in 20 species of Poeciliid fishes (Hewitt et al., 1963) and in hybrids of genus Xiphophorus (Greenberg and Kopac 1965) with the help of paper electrophoresis to find the difference in them. Rabasy (1964) used agar-gel electrophoresis for the separation of protein of 35 fish species.

Comparative muscle myogen electrophorogram study showed virtual constancy and species specific nature of myogen in 50 species of fishes (Tsuyuki et al., 1965). In species of the Petromyzontidae, (Uthe and Tsuyuki 1966) and in Rockfish scorpaenidae (Tsuyuki et al., 1968) muscle myogen pattern was used for the systematics studies.

Studies on muscle protein polymorphism within the genus Tilapia were conducted by Hines and Yashov (1970) and in the genus Merluccius by Jones and Mackie (1970).

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Inter and intra species variation of muscle protein in Japanese Crucian carp was shown in cellulose acetate by Taniguchi and Ishiwatashi (1972) and in starch electrophoresis by Taniguchi and Sakata (1977). Herzberg and Pastear (1975) studied six species of grey mullets in the mediteranean coast of Israel with reference to muscle protein.

Electrophoretic studies on muscle proteins showed distinct patterns in Gobioids of Portonova (Natarajan et al., 1975) in Mullus surmuletus and M. barbatus (Arias and Morales 1977) in frigate tuna Auxis thazard (Yeh and Yang 1977) in Sarpa Salpa and Boops boops (Arias and Morales 1980) and in four species of Sciaenidae (Garcia 1980).

Densitometric analysis was carried out and the crests found were proportional to the protein concentration which was worked out in Anodonta grandis (Saleuddin 1969) in cyprinid fishes (Haen and O' Rourke 1969) and in flat fishes (Menezes 1979).

In crustaceans, work done on this aspect is quite limited. Kannupandi and Paulpandian (1975) studied blood and muscle proteins of crabs and Cole and Morgan (1978) studied muscle protein of the blue crab Callinectes sapidus Rathbun.

Electrophoretic studies on muscle myogens of penaeid prawns like Metapenaeus mutatus, Parapenaeopsis hungerfordi, P. hardwickii, Metapenaeopsis stridulans, M. barbata, Penaeus monodon, P. semisulcatus, Metapenaeus ensis, and Parapenaeopsis affinis were carried out by Idm and Lee(1970) and Lee and Idm (1973). Sriraman and Reddy (1977) found out the characteristic muscle patterns of planktonic juveniles of Penaeus indicus and P. monodon. Kulkarni et al., (1980) separated proteins of four penaeid prawns namely Metapenaeus affinis, M. monoceros, Parapenaeopsis hardwickii and P. stylifera in relation to their sex. Electrophoretic separation in marine prawns Penaeus indicus, Metapenaeus dobsoni, M. monoceros and M. affinis showed specificity in their protein patterns (Thomas 1981). Prathibha(1984) studied in detail protein patterns in different tissues of P. monodon. These studies show that electrophoretic separation of muscle myogen protein patterns confirm and classify the taxonomy of different species. In the present study, using this technique protein patterns of four species of prawns of the genus Metapenaeus namely, M. kutchensis, M. monoceros, M. affinis and M. brevicornis, 3 species of Parapenaeopsis such as P. stylifera, P. sculptilis and P. hardwickii and 5 species of Penaeus such as Penaeus merquiensis, P. penicillatus, P. latisulcatus, P. canaliculatus and P. japonicus were compared.

Results:

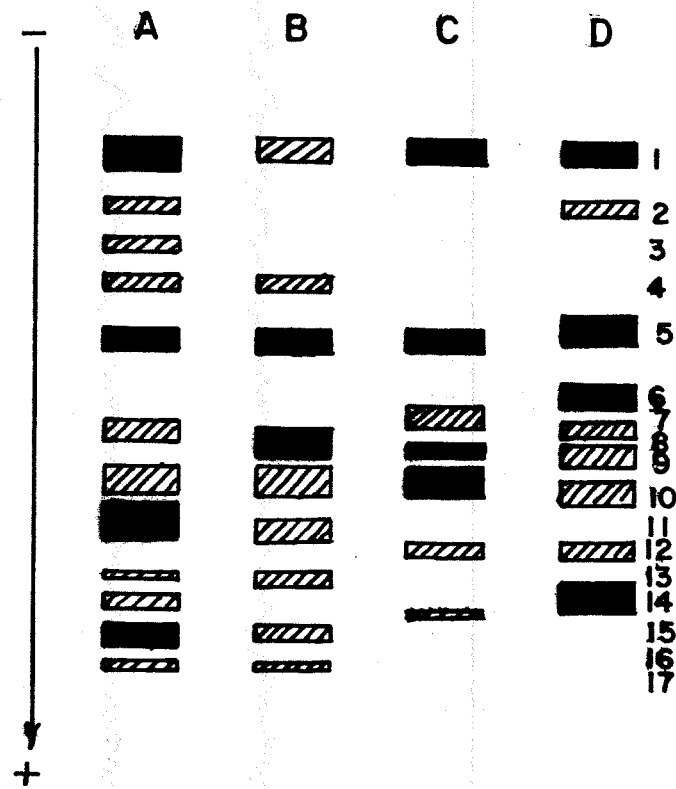
For comparison of each muscle protein band among the four species of Metapenaeus studied here each fraction was allotted a qualifying number obtained according to the electrophoretic mobility position of that particular band, thus the slowest moving and the fastest moving bands receiving the number one and the last number respectively. The bands in between receive the corresponding qualifying numbers. Thus the total number of protein bands present and the allotted numbers for these bands for each species need not be the same (Fig. 2 & 3).

Thus differences in the protein pattern was explained according to their mobility, number of fractions, staining intensities and with the width of each fraction.

Figure 2 & 3 shows the species specific protein patterns of muscle detected in penaeid prawns Metapenaeus kutchensis, M. monoceros, M. affinis, M. brevicornis, Parapenaeopsis sculptilis, P. stylifera and P. hardwickii. These electrophoretic protein patterns help us to identify species which have greater similarity with each other.

Fig. 2. Comparative electrophorograms of abdominal muscle tissues of four Metapenaeus species of prawns. Different shades indicate the intensity of bands.

Fig. 2

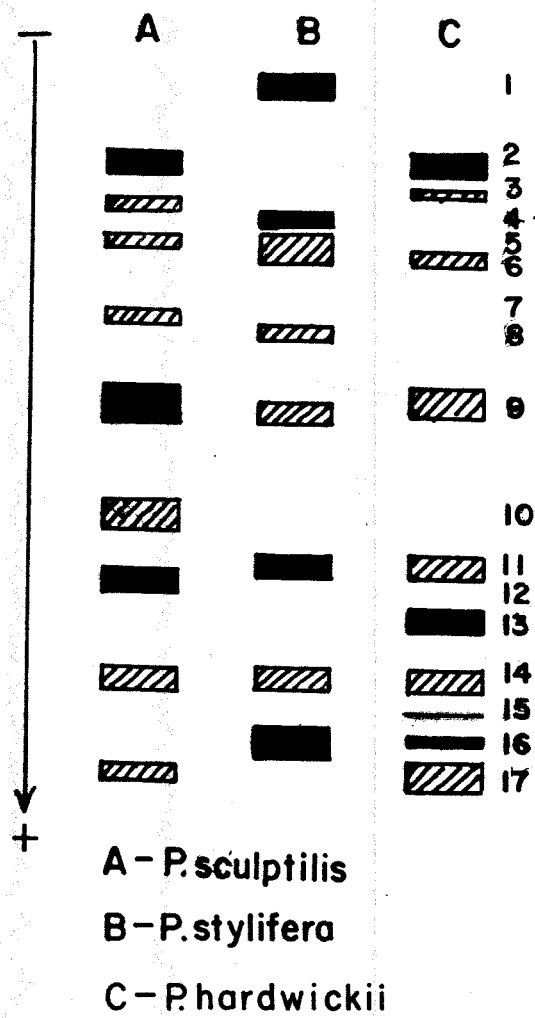


A-M. affinis , B-M. brevicornis

C-M. kutchensis, D-M. monoceros

Fig. 3. Comparative electrophoregrams of
abdominal muscle tissues of three
Parapneustes species.

Fig.3



Metapenaeus species:

First the comparison was made between the species of prawns belonging to Metapenaeus. The protein fractions of Metapenaeus sp. were numbered serially from slowest moving cathodal band to the fastest moving anodal band, thus slowest moving fraction becoming band No.1 and the fastest moving fraction becoming band No. 17 (Fig. 2). Similarity observed in the relative mobility of some of the bands differed by their width and intensity of staining and thus gave a characteristic pattern for that particular species. M. affinis, M. brevicornis, M. kutchensis and M. monoceros showed 12, 9, 7 and 9 muscle protein fractions respectively. The differences in the total number of bands between any of the two species except M. brevicornis and M. monoceros studied here indicated a specific number for muscle protein fractions. Though both M. brevicornis and M. monoceros showed 9 protein fractions each the distinct differences in the electrophoretic mobility, staining intensity and width of certain number of these 9 bands demonstrated a specific pattern for these two species also and thus all the four species showed their own specific muscle protein patterns (Table 21 & 22).

When the common bands found in these species were considered bands No.1, 5 and 10 showed similar relative mobility but their intensity of staining and width of the

Table 21: Relative mobility (RM) with intensity of muscle myogen proteins of *Metapenaeus* species of prawns.

| No. | RM | Intensity |
|--------------------------------------|-------------|-----------|
| <u>Metapenaeus kutchensis</u> | | |
| 1 | 13.3 - 16.7 | XX |
| 2 | 38.3 - 41.7 | XX |
| 3 | 48.3 - 51.7 | X |
| 4 | 53.3 - 55 | XX |
| 5 | 56.7 - 60 | XX |
| 6 | 66.7 - 68.3 | X |
| 7 | 75 - 76.7 | X |
| <u>Metapenaeus monoceros</u> | | |
| 1 | 13.3 - 16.7 | XX |
| 2 | 21.7 - 23.3 | X |
| 3 | 36.7 - 40 | XX |
| 4 | 45 - 48.3 | XX |
| 5 | 50 - 51.7 | X |
| 6 | 53.3 - 56.7 | X |
| 7 | 58.3 - 61.7 | X |
| 8 | 66.7 - 68.3 | X |
| 9 | 71.7 - 75.0 | XX |

Contd...

| No. | RM | Intensity |
|-----|----|-----------|
|-----|----|-----------|

Metapenaeus affinis

| | | |
|----|-------------|----|
| 1. | 13.3 - 16.7 | xx |
| 2 | 21.7 - 23.3 | x |
| 3 | 26.7 - 28.3 | x |
| 4 | 31.7 - 33.3 | x |
| 5 | 38.3 - 41.7 | xx |
| 6 | 50.0 - 53.3 | x |
| 7 | 56.7 - 60.0 | x |
| 8 | 61.7 - 66.7 | xx |
| 9 | 70.0 - 71.7 | x |
| 10 | 73.3 - 75 | x |
| 11 | 76.7 - 80 | xx |
| 12 | 81.7 - 83.3 | x |

Metapenaeus brevicornis

| | | |
|---|-------------|----|
| 1 | 13.3 - 16.7 | x |
| 2 | 31.7 - 33.3 | x |
| 3 | 38.3 - 41.7 | xx |
| 4 | 51.7 - 55.0 | xx |
| 5 | 56.7 - 60.0 | x |
| 6 | 63.3 - 66.7 | x |
| 7 | 70.0 - 71.7 | x |
| 8 | 76.7 - 78.3 | x |
| 9 | 81.7 - 83.3 | x |

Table 22: Summary of muscle myogen patterns of *Metapenaeus* species based on Fig. 2.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | Total No. of bands | Common bands |
|-----------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|--------------------------|-----------------|
| <u>M. affinis</u> | + | + | + | + | + | - | - | + | - | + | + | - | + | + | - | + | + | 12 | 3 |
| <u>M. brevicornis</u> | + | - | - | + | + | - | - | - | + | + | + | - | + | - | - | + | + | 9 | 3 |
| <u>M. kutchensis</u> | + | - | - | - | + | - | + | - | + | + | - | + | - | - | + | - | - | 7 | 3 |
| <u>M. monoceros</u> | + | + | - | - | + | + | - | + | + | + | - | + | - | + | - | - | - | 9 | 3 |

'+' represents presence of the protein band

'-' represents absence of the protein band

band varied to some extent. Similarly in relative mobility of Bands No.2, 8 and 14 showed the resemblance between species M. affinis and M. monoceros. Band No.12 is common for M. kutchensis and M. monoceros (Plate 4).

Parapenseopsis species:

As stated above, the species comparison was made according to total number of band, the relative mobility, intensity of staining and width of the electrophoretic bands.

The species specific total number of bands observed in P. sculptilis, P. stylifera and P. hardwickii, was 9, 8 and 10 respectively (Plate 5). The bands which showed common relative mobility in these 3 species were bands No.6, 9 and 14 (Table 24, 25). At the same time band No. 2 and 17 found in P. sculptilis and P. hardwickii also showed similar fractions, P. stylifera and P. hardwickii expressed similar configuration in band No. 11 and 16. Thus the muscle protein patterns of these 3 species of prawns indicated species specific differences (Fig. 3).

Penaeus species:

Penaeus merquiensis and P. penicillatus: Muscle myogen protein patterns of prawns belonging Penaeus penicillatus and P. merquiensis were compared, using the

Table 24: Relative mobility (RM) with intensity of muscle myogen proteins of *Parapenaeopsis* species of prawns.

| No. | RM | Intensity |
|---|-------------|-----------|
| <u><i>Parapenaeopsis sculptilis</i></u> | | |
| 1 | 15.0 - 18.3 | xx |
| 2 | 21.7 - 23.3 | x |
| 3 | 26.7 - 28.3 | x |
| 4 | 36.7 - 38.3 | x |
| 5 | 46.7 - 51.7 | xx |
| 6 | 61.7 - 65.0 | x |
| 7 | 76.0 - 73.3 | xx |
| 8 | 83.3 - 86.7 | x |
| 9 | 96.7 - 98.3 | x |
| <u><i>Parapenaeopsis stylifera</i></u> | | |
| 1 | 15.0 - 18.3 | xx |
| 2 | 23.3 - 25.0 | xx |
| 3 | 26.7 - 30.0 | x |
| 4 | 38.3 - 40.0 | x |
| 5 | 48.3 - 51.7 | x |
| 6 | 68.3 - 71.7 | xx |
| 7 | 83.3 - 86.7 | x |
| 8 | 91.7 - 95.0 | xx |
| <u><i>Parapenaeopsis hardwickii</i></u> | | |
| 1 | 15.0 - 18.3 | xx |
| 2 | 20.0 - 21.7 | x |
| 3 | 28.3 - 30.0 | x |
| 4 | 46.7 - 50.0 | x |
| 5 | 68.3 - 71.7 | x |
| 6 | 75.0 - 78.3 | xx |
| 7 | 83.3 - 86.7 | x |
| 8 | 88.3 | x |
| 9 | 91.7 - 93.3 | x |
| 10 | 95.0 - 98.3 | x |

Table 25: Summary of muscle myogen patterns of Parapenaeopsis species based on Fig.No.3.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | Total Common No. of bands bands | |
|----------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|---------------------------------------|---|
| <u>P. sculptilis</u> | - | + | - | + | - | + | + | - | + | + | - | + | - | + | - | - | + | 9 | 3 |
| <u>P. stylifera</u> | + | - | - | - | + | + | - | + | + | + | + | - | - | + | - | + | - | 8 | 3 |
| <u>P. hardwickii</u> | - | + | + | - | - | + | - | - | + | - | + | - | + | + | + | + | + | 10 | 3 |

'+'represents presence of the protein band

'-'represents absence of the protein band

gels photographed and scanned in ultra scanner. The electrophoretic fractions obtained were assigned numbers keeping in mind, the number of crests found to correspond to the number of distinct proteins and the areas under the crests were proportional to their concentrations.

For comparative studies of species, total number of protein bands were taken into consideration. P. penicillatus showed 13 muscle protein fractions whereas P. merquiensis showed only 9 protein fractions (Fig. 4). They both shared 8 common bands. The common fractions are No.1, 2, 3, 5, 6, 8, 9 and 14 (Fig. 4) P. penicillatus is found to have 4 additional bands namely the fraction Nos. 10, 11, 12 and 13, whereas in P. merquiensis fraction Nos. 10, 11, 12 & 13 were absent. Fraction No.4 present in P. penicillatus is absent in P. merquiensis. At the same time fraction No. 7 which is present in the later is absent in the former species (Table 27).

According to the width, the bands may be divided into 3 types (a) thicker fractions (b) thinner fractions and (c) smaller fractions. Both species show 5 common thicker fractions which are 1, 2, 3, 5 and 14. When the smaller bands are compared P. penicillatus showed 2 fractions. (Band No. 6 & 8) whereas P. merquiensis showed 3 fractions

Table 27: Summary of muscle myogen patterns of Panæus penicillatus and P. merquiensis based on Fig. 4.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | Total No. of bands | No. of common bands | Deleted band | Additional bands |
|------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|--------------------|---------------------|--------------|------------------|
| <u>P. penicillatus</u> | + | + | + | + | + | + | - | + | + | + | + | + | + | + | 13 | 8 | 0 | 4 |
| | a | a | a | b | a | c | | c | b | b | b | b | b | a | | | | |
| <u>P. merquiensis</u> | + | + | + | - | + | + | + | + | + | - | - | - | - | + | 9 | 8 | 4 | 0 |
| | a | a | a | | a | c | c | c | b | | | | | a | | | | |

a-thicker fractions

b-thinner fractions

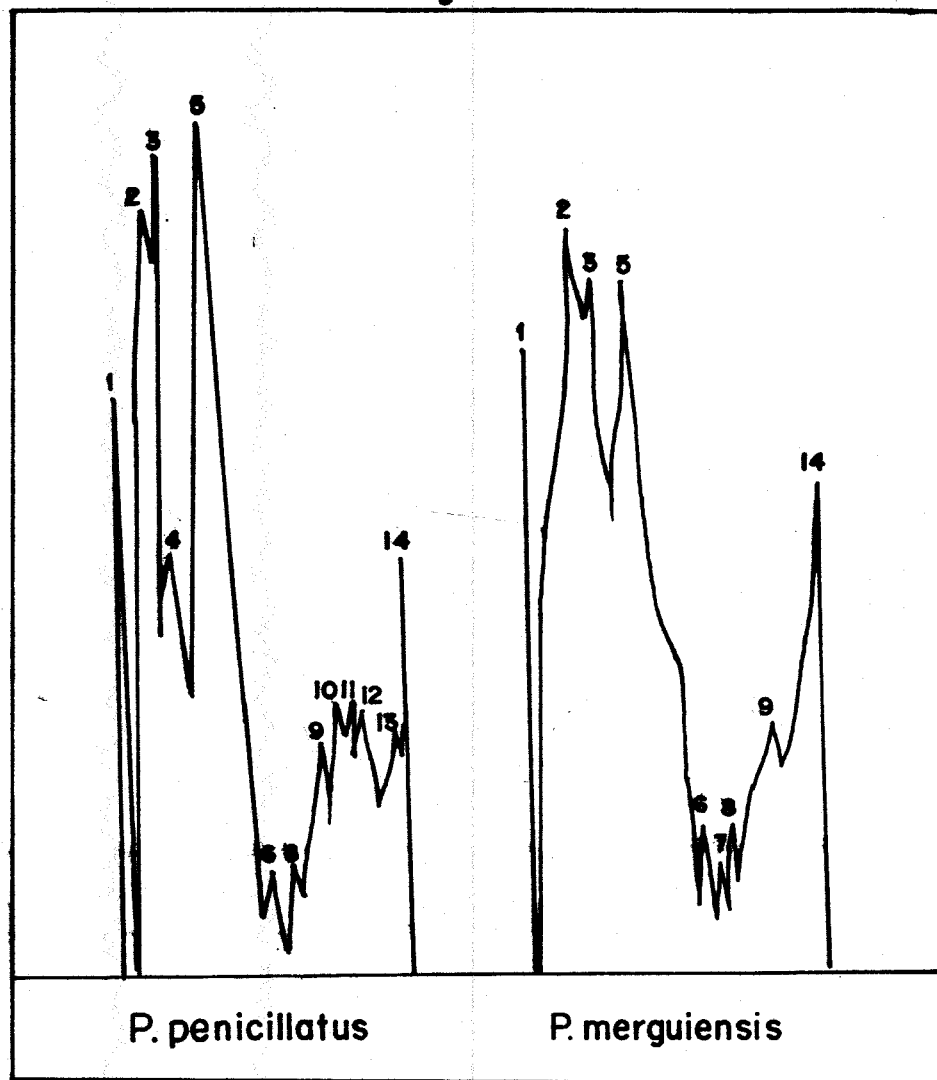
c-smaller fractions

'+' represents presence of the protein band

'-' represents absence of the protein band

**Fig. 4. Comparative scanned pattern of abdominal
muscle tissues of Penaeus penicillatus and
Penaeus merquiensis.**

Fig. 4



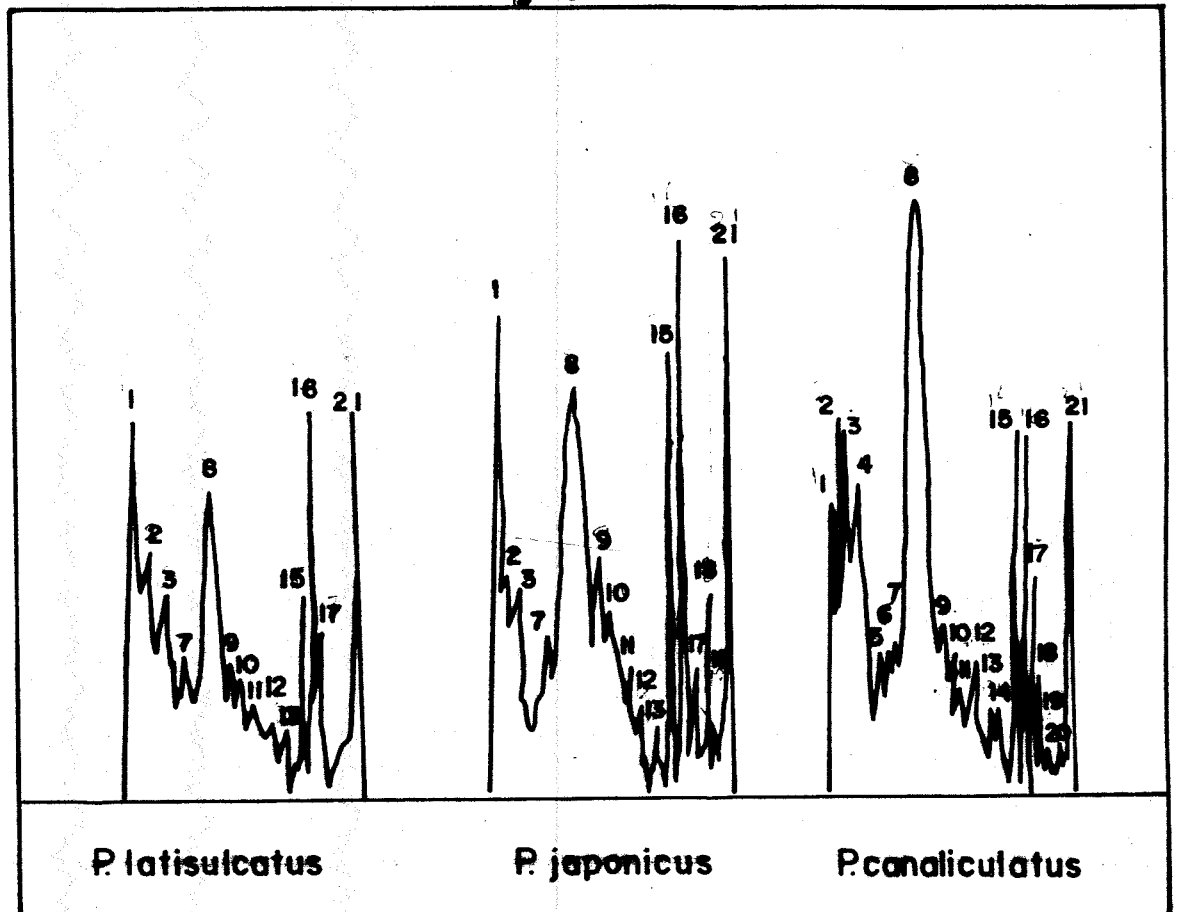
(Band No. 6, 7 and 8). When the second type of thinner bands are compared P. penicillatus showed band Nos. 4, 9, 10, 11, 12 and 13 and P. merquiensis showed band No. 9.

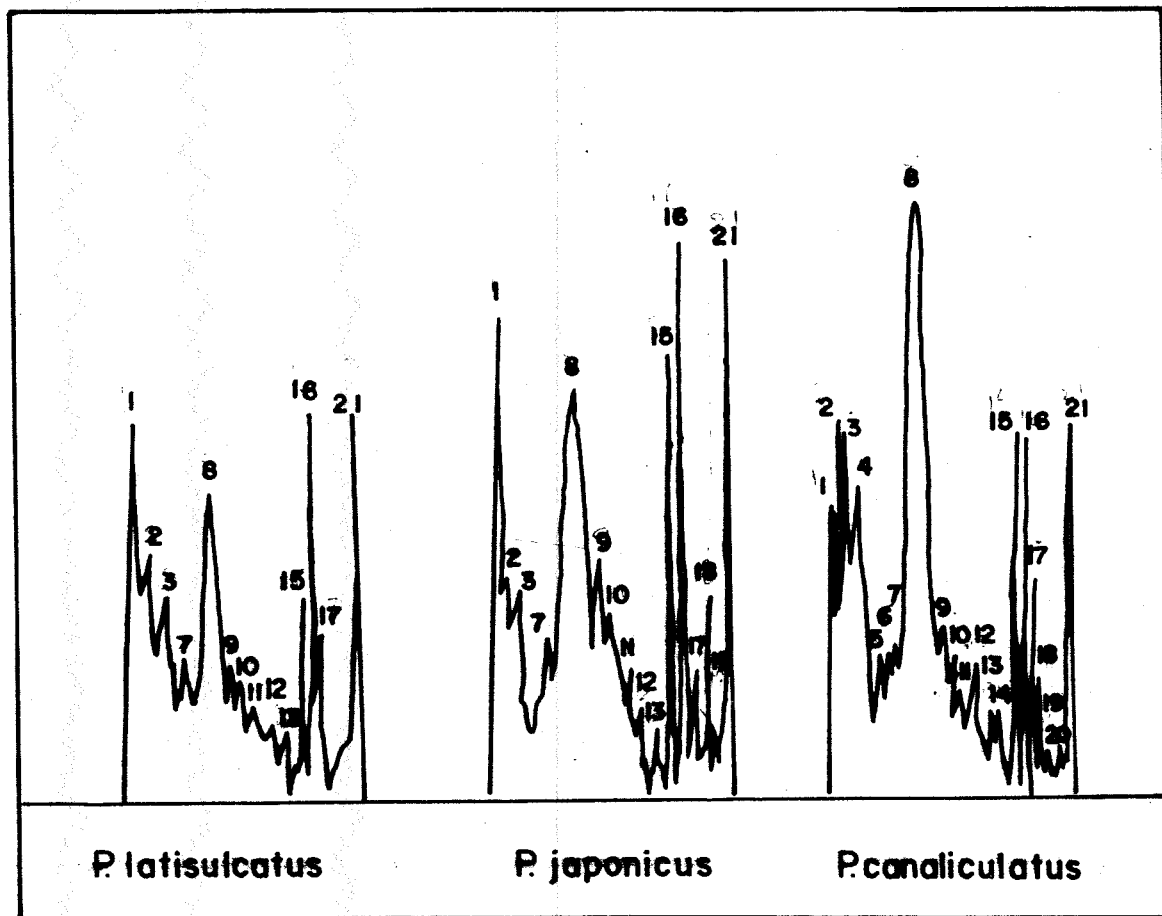
Penaeus latisulcatus, P. japonicus and P. canaliculatus Muscle myogen proteins of closely related penaeus species of prawns like Penaeus latisulcatus, P. japonicus and P. canaliculatus were electrophoretically separated and thus biochemically distinguished from each other. Scanned pattern of this muscle myogen proteins is given in Fig. No. 5. As explained in P. merquiensis and P. penicillatus the bands were numbered according to the crests formed been which represent the concentration of protein bands separated on the gel.

Analysis of electrophorogram revealed total of 14 bands in P. latisulcatus, 16 bands in P. japonicus, and 21 bands in P. canaliculatus. Fourteen common bands (Band No. 1-3, 7-13, 15-17 and 21) were observed in these three species of penaeids. All common bands were seen in P. canaliculatus whereas 5 bands (Band No. 4-6, 14 & 20) were absent in P. japonicus and 7 bands (bands No. 4-6, 14, 18-20) were absent in P. latisulcatus. Thus P. japonicus and P. latisulcatus showed their distinctive distinguishing characters by the absence of the above mentioned bands.

Fig. 5. Comparative scanned pattern of abdominal muscle tissues of Penaeus latisulcatus, Penaeus japonicus and Penaeus canaliculatus.

Fig. 5





Scanned pattern observed can be divided into 3 groups. Band No.1-4 forms first group, Band No.5-14 forms second group and Band No.15-21 forms third group. When the comparison was made within first group of bands, band No.1-3 is seen in all the 3 species of prawns but Band No.4 is observed only in P. canaliculatus, P. japonicus and P. latisulcatus are found to be devoid of Band No.4. When group II type of bands were analysed all the 10 bands were present in P. canaliculatus contradictory to this Band No.5, 6 and 14 were absent in P. latisulcatus and P. japonicus. Thus group II showed only 8 bands in P. latisulcatus and P. japonicus. All the Group III Bands were present in P. canaliculatus but band Nos. 18-20 and band No. 20 were absent in P. latisulcatus and P. japonicus. According to the width of the band the scanned pattern is divided into 3 types. Thicker bands, thinner bands and smaller bands thus P. latisulcatus has one thicker band (band No.8) 4 thinner bands (band Nos.1, 15, 16, 17 and 21) and 8 smaller bands (Band Nos. 2, 3, 7 9-13) P. japonicus showed 2 thicker bands (band No.1 & 8), 4 thinner bands (15, 16, 18 and 21) and 10 smaller bands (band No.2, 3, 7, 9-13 17 & 19). P. canaliculatus expressed one thicker band (band No. 8), 9 thinner bands and 11 smaller bands (band Nos. 5-7, 9-14, 19-20). In this way all these three species which have morphology expressed species specific differences in their protein patterns. (Table 29)

Table 29: Summary of muscle myogen pattern of Penaeus latisulcatus, P. isponicus and P. canaliculatus based on Fig. 5.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | Total No. of bands | Number of common bands | Deleted bands | Additional bands. |
|-------------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|-----------------------|------------------------------|------------------|----------------------|
| <u>P. latisulcatus</u> | + | + | + | - | - | - | + | + | + | + | + | + | + | - | + | + | + | - | - | - | + | 14 | 14 | 7 | 0 |
| <u>P. isponicus</u> | + | + | + | - | - | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | 16 | 14 | 5 | 5 |
| <u>P. canaliculatus</u> | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 21 | 14 | 0 | 7 |

'+' represents presence of the protein band

'-' represents the absence of the protein band.

Discussion:

Accurate identification of organism at species level is a pre-requisite for the progress of scientific research in any field of biological science. Study of natural differences in the morphometrics and meristics has always been a popular traditional method for establishing species identity. Nevertheless, overlapping nature of morphological and meristic characteristics qualified among the animals to be identified may cause practical difficulties even for the expert taxonomist. Such inherent taxonomic problems cannot be easily solved by morphological comparisons alone (Wright 1966). A certain amount of innate plasticity of morphometrics and meristic characters present in fish has caused difficult taxonomic problems (Wilkins 1967). Hence, biologists began to introduce variety of modern experimental techniques developed in their period of research, particularly, in the field of medical sciences for improving the method of species identification.

Thus muscle protein of fishes were compared using classical Tiselius Technique of electrophoresis (Connel 1953, a, b Dingle et al., 1955), paper electrophoresis (Hewitt et al., 1963) and agar gel electrophoresis (Rabaey 1964). Buzzati-Traverso and Rechnitzer (1953) introduced

the use of chromatographic techniques in taxonomic studies. High resolution starch gel electrophoresis method established by Smithies (1955) found immediate application in various fields of biological research including fisheries. The efficiency of gel electrophoresis to separate and resolve biochemical properties of an individual or experimental animal at molecular level proved of immense help in solving even inherent taxonomic problems. The relationships of DNA molecule with structural proteins as explained by Crick (1963) and Nirenberg et al., (1963) enabled the biologist to interpret the electrophoretically separated protein molecules in terms of genetics of the experimental animal, bringing taxonomy to most natural level. The taxonomists could label individual inherent differences at species and higher level of classification and corroborate and verify the traditional method of species differentiation. Thus the problem of taxonomic status of North Atlantic Sebastes fish was solved using agar gel electrophoresis (Altukhov and Nefyodov 1968). There are examples of unrecognized species being detected through the use of electrophoretic and biochemical techniques as in fish (Sage and Selander, 1975) and Snails, (Woodruff, 1978). Lester (1980) demonstrated biochemical genetic differences among P. aztecus, P. duorarum and P. setiferus.

Muscle is an important body tissue of all animals. It is commonly used for electrophoretic and biochemical investigations. Its protein is known as myogen. For comparative studies muscle myogen protein patterns was widely employed on fishes.

As the crustacean group of organism, particularly, prawns possess many overlapping and similar body characters, their accurate identification at species and even in generic level is difficult. Due to lack of easily observable species specific characteristics, species status is often subject to changes as seen in the case of P. subtilis and P. notialis each having related subspecies in Gulf of Mexico (Perez Farfante 1978). The Indian prawns M. kutchensis has a close resemblance with M. monoceros and M. affinis, particularly males (Table 20). Confusing taxonomic status of M. necopinans and M. mutatus was caused as they were probably synonymous of M. affinis (George 1979).

Though electrophoresis is a powerful analytical tool for solving problems of species identification, information on its application in crustacean group of organisms are limited. Lim and Lee (1970) separated muscle proteins of Metapenaeus mutatus, Parapenaeopsis hungerfordi, P. hardwickii, Metapenaeopsis stridulans, M. barbata, Penaeus

Table 20: Morphological variation between M. kutchensis, M. monoceros, M. affinis and M. brevicornis

| <u>M. kutchensis</u> | <u>M. monoceros</u> | <u>M. affinis</u> | <u>M. brevicornis</u> |
|---|---|--|--|
| No expod on 5th pereopod; pleuro-branch on 7th thoracic somite present. | No expod on 5th pereopod; pleuro-branch on 7th thoracic somite present. | No expod on 5th pereopod; pleuro-branch on 7th thoracic somite present. | No expod on 5th pereopod; pleuro-branch on 7th thoracic somite present. |
| Ischial spine on 1st pereopod distinct. | Ischial spine on 1st pereopod distinct. | Ischial spine on 1st pereopod distinct. | Ischial spine on 1st pereopod small or absent. |
| Posterior extension of the anterior median thelycal plate not bound laterally by oval plate on either side; distomedian petasmas projections not overlying lateral projections. | Lateral thelycal plates with salient and parallel earshaped lateral ridges; distomedian petasmas projections hood-like. | Anterior thelycal plate longitudinally grooved, wider posteriorly than anteriorly, distomedian petasmas projections crescent-shaped. | Posterior part of rostrum with distinctly elevated crest; basal spine on male 3rd pereopod simple, apical petasmas filaments slender, slightly converging thelycum with large anterior and small lateral plates. |

Source George (1979).

monodon, P. semisulcatus, Metapenaeus ensis and Parapenaeopsis affinis using cellulose acetate electrophoresis.

Later very high resolution giving polyacrylamide disc gel electrophoresis introduced by Davis (1964) was employed for separating tissue proteins for species identification of juveniles of Penaeus indicus and P. monodon (Sriraman and Reddy 1977), Metapenaeus affinis, M. monoceros, Parapenaeopsis hardwickii and P. stylifera in relation to sex (Kulkarni et al 1980) P. indicus, Metapenaeus dobsoni, M. monoceros and M. affinis (Thomas 1981) and P. monodon (Prathibha 1984).

The aim and objective of the present investigation was to discover natural and reliable species specific characteristics of selected species of Indian prawns like Parapenaeopsis stylifera, P. sculptilis, P. hardwickii, M. kutchensis, M. monoceros, M. affinis and M. brevicornis using polyacrylamide gel electrophoresis. All these species tested here can be distinguished easily on the basis of differences in the total number of muscle protein bands, their electrophoretic mobility and even staining intensity (Fig. No.2 & 3).

Metapenaeus species:

The present study has given 9 bands in M. monoceros, 12 in M. affinis, 9 in M. brevicornis and 7 bands in

M. kutchensis, from a location in Bombay on the north west coast of India showing a species specific nature in the number of bands. Total number of bands observed in different species of Metapenaeus by previous authors is 8 bands in M. dobsoni 11 fractions in M. affinis and 7 bands in M. monoceros by Thomas (1981), 8 bands each in M. mutatus, M. stridulans, M. barbata and M. ensis by Lim and Lee(1970), The latter study does not show any species specificity in the number of bands, all the species studied in the same genus showing similar bands in relation to number. However the study of Thomas (1981) do show difference in number of bands between M. monoceros, M. affinis and M. dobsoni, being 7, 11 and 8 bands in the three species respectively. However the present result of 9 bands in M. monoceros does not seem to agree with the observation of 7 bands by Thomas (1981). In M. monoceros also there is a difference of 1 band. The reason which could be attributed to this difference in the number of bands in the same species appears to be either geographic variation, the location of specimens collected being wide apart or the methodology applied in the finer analysis and standardisation. In the case of M. brevicornis which is preserved in 2% phenoxy ethanol showed 7 bands (Lim and Lee 1970) in cellulose acetate gel whereas M. brevicornis tested here showed 9 bands in acrylamide gel

which is known to give better resolution than cellulose acetate gel. Geographical differences in the species tested here may also account for the variation in the total number of band.

Since the specimens are collected in the immature gonad stage there is no difference observed in the male and female specimens analysed. Difference in the male and female sex is shown by Lim and Lee (1970) in the case of M. mutatus with 8 bands in female and 7 bands in male and M. ensis male with 8 bands female with 7 bands but M. brevicornis and M. stridulans which showed 7 and 8 band in both male and female specimens.

According to the relative mobility the comparison between Metapenaeus mutatus, M. stridulans and M. barbata. showed 3 common bands (Lim and Lee 1970) that shows probable generic relationship. M. affinis, M. brevicornis, M. kutchensis and M. monoceros expressed 3 common bands (Band Nos. 1, 5 & 10) indicating their probable common generic relationship and the characteristic feature for the identification of this genus.

The comparison made between bands No.2, 8 & 14 showed the relationship between M. affinis and M. monoceros Band No. 4, 11, 13, 16 & 17 showed the similarity of protein pattern

seen between M. affinis and M. brevicornis Band No. 9 showed the similar mobility existed between M. brevicornis, M. kutchensis and M. monoceros. Band No. 12 expresses similarity between M. kutchensis and M. monoceros (Fig.2).

In this way above differences and similarities expressed by protein fractions can be applied for the biochemical identification of these species besides their morphological identification. Their species specific nature can also be utilized as a tool for the identification of these species and also to distinguish among themselves.

Parapenaeopsis species:

Earlier workers has pointed out the morphological characteristics of the species like P. hardwickii, P. stylifera and P. sculptilis and detail. (Rao 1970, George 1975, Fischer and Bianchi 1984) (Table 23).

To find out additional plausible evidence by means of biochemical analysis to reveal the species specific and distinguishing characters between these three species, electrophoretic studies on muscle myogen patterns were analysed. Biochemical systematics of this genus was carried out already in P. hungerfordi, P. hardwickii (Lim and Lee 1970), P. hardwickii and P. stylifera (Kulkarni et al., 1980).

Table 23: Morphological variation between P. hardwickii, P. sculptilis, and P. stylifera.

| <u>P. hardwickii</u> | <u>P. sculptilis</u> | <u>P. stylifera</u> |
|---|---|--|
| 3rd pereopod without epipodite. | 3rd pereopod without epipodite. | 3rd pereopod without epipodite. |
| Antennular flagella 0.7 length of carapace or longer; movable lateral spines present on telson. | Antennular flagella 0.5-0.6 length of carapace movable lateral spines absent on telson. | -- |
| Petasma with pair of short spout-like distolateral projections and pair of cup like distal projections. | Distomedian projections of petasma large and flare out laterally anterior thelycal plate separated from the posterior sternal plate by a short intervening space. | Petasma long with distolateral projections divergent; appendix maxillina with distolateral projection. |

Source: George (1979)

In the present study P. stylifera, P. hardwickii and P. sculptilis showed 8, 10, and 9 bands. Besides the total number of bands these three species vary by the electrophoretic mobility, staining and the width of the bands.

P. hardwickii analysed by Lim and Lee (1970) showed 8 bands and the present study showed 10 bands. This may be due to the usage of polyacrylamide gel in the present study which is superior to cellulose acetate employed by former and also probable geographical variation in the species.

Common bands seen in Parapenaepsis genus by the present study showed their generic similarity, P. hungerfordi and P. hardwickii (Lim and Lee 1970) expressed 7 common bands whereas in the present study P. sculptilis, P. stylifera and P. hardwickii expressed only 3 common bands in band No. 6, 9 & 14. The wide differences observed in these above mentioned studies may be due to the better separation using polyacrylamide gel and the geographical variation expressed within these species. This also reveals the greater differences within these species occurring in these area. Band number 2 and 17 found to have same relative mobility between the species P. sculptilis and P. hardwickii and band No. 11 and 16 expressed closeness between P. stylifera and P. hardwickii. With these electrophorograms patterns observed it is very easy

to distinguish these three species. The patterns observed also is species specific and the specific differences and the closeness between these three species were clearly seen.

Penaeus species:

P. penicillatus and P. merquiensis

Morphologically P. penicillatus and P. merquiensis are very closely allied, the only important difference being in the length of the dactyl of 3rd maxilliped of adult males. Thus it is very difficult to distinguish the two species when they are smaller in size. Therefore these two species were selected to study their muscle protein variation in order to use it as a taxonomic tool for identifying the 2 species.

Earlier workers used densitometric reading for the analysis of isoenzyme patterns of Anodonta grandis (Saleuddin 1969) serum patterns of flat fishes (Maria 1979) and muscle proteins of five Cyprinids (Haen and O'Bourke 1969b). Likewise here also the gels were scanned and the results were interpreted.

Polyacrylamide gel which gave good resolution was used as medium here for separation of proteins as reported in the species identification of P. indicus, P. monodon

(Sriraman and Reddy 1977), P. indicus (Thomas, 1981) and P. monodon (Prathibha 1984).

Differences in the total number of muscle protein bands namely 10 and 11 in P. indicus and P. monodon respectively (Sriraman and Reddy 1977) 8 bands in P. indicus (Thomas 1981) 7 bands in P. monodon, 9 bands in P. semisulcatus (Lim and Lee 1970) and 16 bands in P. monodon (Prathibha 1984) demonstrated species specific pattern of muscle proteins (Table 26).

Lim and Lee (1970) reported the presence of five bands of common electrophoretic mobility between P. semisulcatus and P. monodon as indicative of their close relationship at generic level. The present observation of eight common bands between P. penicillatus and P. merguensis may also suggest greater generic relation between these two species studied here whereas four bands 10, 11, 12 and 13 present only in P. penecillatus demonstrates the species specific differences of these same two species (Fig. 4).

Lim and Lee (1970) reported only 7 muscle protein bands in P. merguensis whereas 9 bands were obtained in the present study. This significant difference in the total number of bands as revealed in the above comparison may be due to slight difference in methodology adopted in

Table 26; Details of Muscle myogen patterns observed in different species of prawns.

| Species | Total number of bands |
|-------------------------------|------------------------------|
| <u>P. sculptilis</u>* | 9 |
| <u>P. stylifera</u>* | 8 |
| <u>P. hardwickii</u>* | 10 |
| <u>M. kutchensis</u>* | 7 |
| <u>M. monoceros</u> | 9 |
| <u>M. affinis</u>* | 12 |
| <u>M. brevicornis</u>* | 9 |
| <u>P. indicus</u>** | 10 |
| <u>P. monodon</u>** | 11 |
| <u>P. monodon</u>*** | 16 |

*** Present study**

**** Sriraman and Reddy 1977**

***** Prathibha 1984**

Table 26: Details of Muscle myogen patterns observed in different species of prawns.

| Species | Total number of bands |
|-------------------------------|------------------------------|
| <u>P. sculptilis</u>* | 9 |
| <u>P. stylifera</u>* | 8 |
| <u>P. hardwickii</u>* | 10 |
| <u>M. kutchensis</u>* | 7 |
| <u>M. monoceros</u> | 9 |
| <u>M. affinis</u>* | 12 |
| <u>M. brevicornis</u>* | 9 |
| <u>P. indicus</u>** | 10 |
| <u>P. monodon</u>** | 11 |
| <u>P. monodon</u>*** | 16 |

*** Present study**

**** Sriraman and Reddy 1977**

***** Prathibha 1984**

the respective studies. The muscle tissue tested by Lim and Lee (1970) was preserved in 2% phenoxy-ethanol whereas the muscle in the present study was taken and tested from a fresh specimen. The effect of different geographical regions of the species may also account for the observed differences in the total number of proteins.

The important difference in the total number of muscle protein fractions detected between P. penicillatus and P. merguensis in the present study clearly indicated the taxonomic identity of these two species, the number of fractions being 13 and 9 respectively.

The significant species specific muscle protein pattern differences between P. penicillatus and P. merguensis revealed in the present study proves the efficiency of electrophoretic techniques in solving the problems of species identity of morphologically very similar species of prawns.

P. latisulcatus, P. canaliculatus and P. japonicus

Morphologically P. latisulcatus, P. canaliculatus and P. japonicus closely resemble each other. Important morphological differences observed are given in table No.28. Because of these overlapping characters, ambiguous species

Table 28: Morphological variation between P. latisulcatus P. japonicus and P. canaliculatus.

| <u>P. latisulcatus</u> | <u>P. japonicus</u> | <u>P. canaliculatus</u> |
|--|---|-------------------------|
| 1. Telson armed usually with 3 pairs of spinules. | 1. Telson armed usually with 3 pairs of spinules. | 1. Telson unarmed. |
| 2. Adrostral sulcus as wide as postrostral carina. | 2. Adrostral sulcus narrower than postrostral carina. | -- |
| 3. Anterior plate of thelycum bifid at the apex. | 3. Anterior plate of thelycum rounded at the apex. | -- |

Source: George (1979)

nature exists during their developmental stage. Thus these species were selected for discovering possible biochemical genetic differences which may exist in their muscle proteins.

Scanned patterns observed in these species also showed 14 common bands (Band No.1-3, 7-13, 15-17 and 21) expressing close ancestral relationship of P. latisulcatus, P. canaliculatus and P. japonicus. Present study revealed a total of 14 bands for P. latisulcatus due to the deletion of bands Nos. 4, 5, 6, 14, 16, 18, 19 & 20 from P. canaliculatus. Deletion of the band Nos. 4, 5, 6 14 & 20 when compared with P. japonicus showed its biochemical difference from P. japonicus.

Intra species variation studies on P. latisulcatus was carried out by Richardson (1982b) Mulley and Latter 1980 used P. latisulcatus to find out the evolutionary relationships within a group of thirteen species of Penaeid prawns. and De Matthaeis et al., (1983) worked on the genetic difference between P. japonicus and P. kerathurus.

Characteristic species specific patterns observed using muscle myogen patterns can be used to solve the species identity in addition to the morphological characters.

The individual differences detected here are indicative of species specific nature of muscle myogen electrophoretic

fractions as established and reported in several other species of prawns (Table 30). These informations can now form a strong basis for understanding of these species at biochemical genetic level and further help in any hybridization and genetic manipulation studies desirable for scientific management of these valuable cultivable resources.

Table 30: Groupwise comparison of muscle myogen patterns in different Penaeid prawns.

| | | | | |
|----------------------------|---|---|----|----|
| <u>P. indicus</u> * | 4 | 3 | 3 | 10 |
| <u>P. monodon</u> * | 3 | 4 | 4 | 11 |
| <u>P. monodon</u> ** | 8 | 5 | 3 | 16 |
| <u>P. penicillatus</u> *** | 5 | 6 | 2 | 13 |
| <u>P. merguensis</u> *** | 5 | 1 | 3 | 9 |
| <u>P. latisulcatus</u> | 1 | 5 | 8 | 14 |
| <u>P. japonicus</u> | 2 | 4 | 10 | 16 |
| <u>P. canaliculatus</u> | 1 | 9 | 11 | 21 |

* Sriraman & Reddy 1977 (According to electrophorogram)

** Prathibha 1984 (According to electrophoregrams)

*** Present study (According to scanning)

CHAPTER V

ONTOGENETIC VARIATION

Resume of literature

Development is the process by which a single fertilized cell becomes a complex organism. Ontological development leads to morphological changes in different organisms due to corresponding variation in the metabolic pathways and gene regulation patterns during the process of their growth. Isoenzymes and other protein types being specific gene products are efficient markers of cell types (Rider 1980). Hence these protein types can be used as control to assess the changes which occur during cellular protein enhancement during developmental changes. Thus observed differences in the isoenzyme activity can be related to developmental changes in isoenzyme synthesis. These changes can also be identified qualitatively by using electrophoretic separation of isoenzyme.

Artemia was intensively studied to understand the specific developmental changes occurring in them. (Hentschel and Tata 1976, Bagshaw and Warner 1979 and Clegg and Conte 1980). Ontogenetic changes were studied using isoenzymes patterns of Homarus americanus (Hedgecock

et al,1975) and in crabs (Gooch 1977, Morgan et al,1978 and Kannupandi 1980).

Lester (1980) using electrophoresis pattern of isoenzyms identified juvenile shrimps of different geographical areas. Different species of prawns has their own characteristic pattern of development.

In Penaeus species Lester and Cook (1987) compared gene expression in different developmental stages of four species of prawns viz; P. aztecus, P. setiferus, P. stylifrostris and P. Vannamei). Rizzotti et al, (1977) and Zoarces (Hjorth 1974) showed tremendous ontogenetic changes in haemoglobin of elver and adult stages of Anquilla anquilla.

Lactate dehydrogenase isoenzyme during ontogeny is visualized in two salmonids Salmo salar and S. trutta, (Kunz 1975) Palaemon serratus, (Thebault and Bernicard 1978) in Coho Salmon, (Marquez 1978) and in Liza parsia (Parag 1984). The same isoenzyme pattern in heart tissue expressed a change from predominantly LDH-5 to a predominant and of isoenzyme LDH-1 during the development of an embryo of 9 days before birth to the adult mouse (Market 1983).

Alkaline phosphatase isoenzyme noticed in Drosophila melanogaster, showed changes, during its developmental stages like young larva, instar larva, pupa and adult (Beckman and Johnson 1964). Again Morgan et al, (1978) in Xanthid crab, Mary (1985) in Mugil cephalus and Lester and Cook (1987) in penaeid prawns showed the ontological changes in this enzyme.

Esterase isoenzyme pattern (Paul and Fottrell 1961) found in foetal human tissue resemble those of adults but Blanco and Zinkham (1966) reported an increase in both the number and activity of isozymes during development. Hunter et al, (1964) described enzyme changes in development of liver and kidney in the foetal and weanling mouse.

Esterase patterns of various Cavian tissues (Holmes and Masters 1967) show marked changes in the liver, kidney, and intestine.

Various other workers like Flowerdew (1976) in the Cirripede Balanus balanoides Gooch (1977) in crabs, Morgan et al, (1978) and Kannupandi (1980) in Xanthid Crabs and Lester and Cook (1987) in prawns of Penaeus species had worked on esterase to show their changes during development.

General protein patterns during ontogenetic development expressed gradual changes in Salmon (Nyman, 1967 Battacharya and Alfred, 1982) and in mullet (Herzberg and Pasteur 1975). Sriraman and Reddy (1977) showed changes in protein patterns during development of planktonic juveniles and adults of P. indicus and P. monodon Kannupandi (1980) in Xanthid crab, Prathibha (1984) in P. monodon and Lester and Cook (1987) in prawns P. aztecus, P. setiferus, P. stylirostris and P. vannamei.

Malate dehydrogenase enzyme changes during different stages of development is shown in crabs by Gooch (1977) and Morgan et al., (1978) and in plants (Rider 1980).

Aldehyde oxidase enzyme showed ontogenetic variation in penaeid spp. of prawns (Lester and Cook 1987).

Alcohol dehydrogenase showed ontogenetic changes which was correlated with liver maturation in the Brachydanio nigrofaciatus (Frankel 1981).

To find out the distinct biochemical variations taking place in the larval stages in the development of prawn, these were analysed using electrophoresis to get additional in sight for the larval identification of some species (Lester 1980) and to find out the changes taking place during its ontogenetic development.

The present work was aimed at finding out the isoenzyme expression and its specificity in different stages of development of P. indicus. It would help to attain characteristic band pattern in different stages due to the changes in the gene expression for the identification of larvae of P. indicus from other larvae.

There exist an inverse relationship between larval dispersal and the extent of population genetic differentiation (Gooch et al, 1972). To find out the population structure in marine environment nowadays efforts are focussed on species which have planktonic development stage with larval dispersal capacity (Burton 1983).

As these studies are very limited with reference to Indian prawns, the present investigation was undertaken to reveal probable ontological changes in the isoenzyme patterns of the prawn P. indicus.

Results:

Separation of isoenzymes using polyacrylamide gel electrophoresis revealed different enzyme patterns in different larval stages in the white prawn P. indicus collected from Narakkal Prawn Culture Laboratory, Narakkal. Various enzymatic and general proteins tested here showed stage specific protein patterns. Isoenzymes of acid phosphatase and aldehyde oxidase were studied in Protozoa, mysis and post larval stages, while, alcohol dehydrogenase, malate dehydrogenase, octanol dehydrogenase, esterase, and general protein patterns were analysed in protozoa, post-larva, different size groups of juveniles namely 20-30 mm, 40-50 mm, 70-80 mm and adults (90-120 mm).

Isoenzyme patterns detected in each of the above mentioned enzymes of different stages are given in Fig. Nos. 6-12. Exact position of all the bands obtained were drawn according to their relative mobility value. Bands were given serial numbers starting from the cathodal end towards the anodal end and in the order of increasing electrophoretic mobility.

Acid phosphatase:

Acid phosphatase isoenzyme patterns were detected in different stages of the life cycle of Penaeus indicus namely

protozoa, mysis, postlarvae, three different size range of juveniles namely 20-30 mm. 40-50 mm, 70-80 mm and adult (90-120 mm) (Plate 6).

Band Nos. 7 and 8 are present only in protozoa stage. Band No.6 is absent in protozoa and mysis and appears in post-larva, in all juvenile and adult stages. Band No.5 appears in juvenile I, II and III stages and disappears in other stages. Though Band No.4 is present in protozoa, mysis and post-larva stages, it is absent in all juvenile and adult stages. Band No.3 is found only in post-larva and juvenile stage I. Band No.2 is found to be expressed only in protozoa and mysis stages. Band No.1 is exhibited only in mysis stage. Band Nos. 1, 2, 4, 7 & 8 are expressed in larval stages only, whereas Band No.5 and 6 are found mainly in juvenile and adult stages (Fig. No.6). Thus the Isoenzymes of Acid phosphatase are found to be expressed in different electrophoretic mobility and in different numbers, indicating a stage-specific pattern for the enzyme (Table 31 & 32).

Aldehyde oxidase:

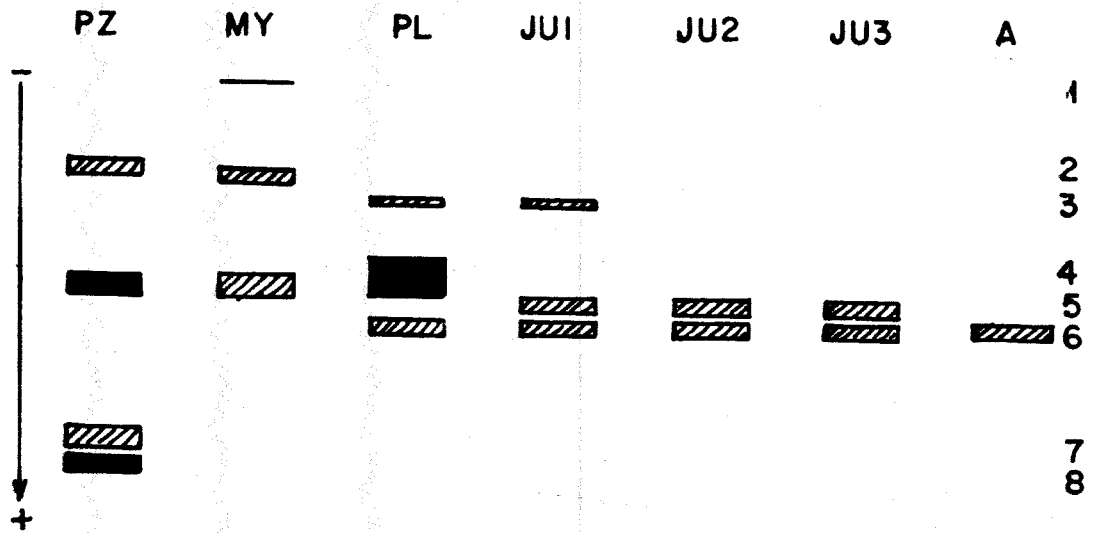
Aldehyde oxidase Isoenzyme patterns of different stages of Penaeus indicus mentioned earlier were found out (Fig. No.7) Protozoa stage had 4 distinct bands (Band No.3, 6, 8 & 9). Mysis showed 2 bands (Band No. 4 & 5), post-larva

Table 31: Relative mobility (RM) with intensity of Acid phosphatase bands separated in Penaeus indicus.

| Stage | Band Nos. | RM Value | Intensity |
|--------------------|-----------|-------------|-----------|
| Protozoaea | 2 | 11.7 - 13.3 | x |
| | 4 | 26.7 - 30.0 | xx |
| | 7 | 46.7 - 50.0 | x |
| | 8 | 51.7 - 53.3 | xx |
| Mysis | 1 | 1.7 - 3.3 | x |
| | 2 | 13.3 - 15.0 | x |
| | 4 | 26.7 - 30.0 | xx |
| Postlarvae | 3 | 16.7 - 18.3 | x |
| | 4 | 25.0 - 30.0 | xx |
| | 6 | 33.3 - 35.0 | x |
| 20-30mm (Juvenile) | 3 | 15.7 - 18.3 | x |
| | 5 | 30.0 - 31.7 | x |
| | 6 | 33.3 - 35.0 | x |
| 10-50mm (Juvenile) | 5 | 30.0 - 31.7 | x |
| | 6 | 33.3 - 35.0 | x |
| 70-80mm (Juvenile) | 5 | 30.0 - 31.7 | xx |
| | 6 | 33.3 - 35.0 | xx |
| 90-120mm (Adult) | 6 | 33.3 - 35.0 | x |

**Fig. 6. Ontogenetic variation of acid phosphatase
enzyme in Penaeus indicus.**

Fig. 6



PZ-Protozoa, MY-Mysis, PL-Post larva

JU1-20-30mm, JU2-40-50mm, JU3-70-80mm

A-90-120mm

Table 32: Summary of acid phosphatase patterns of Penaeus indicus based on Fig. 6.

| Stages | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total No. of bands |
|--------------------|---|---|---|------|---|---|---|------|--------------------------|
| Protozoa | - | + | - | ++ | - | - | + | (++) | 4 |
| Mysis | + | + | - | + | - | - | - | - | 3 |
| Post-larvae | - | - | + | (++) | - | + | - | - | 3 |
| 20-30mm (Juvenile) | - | - | + | - | + | + | - | - | 3 |
| 40-50mm(") | - | - | - | - | + | + | - | - | 2 |
| 70-80mm(") | - | - | - | - | + | + | - | - | 2 |
| 90-120mm (Adult) | - | - | - | - | - | + | - | - | 1 |

'+' represent the presence of the Isozyme

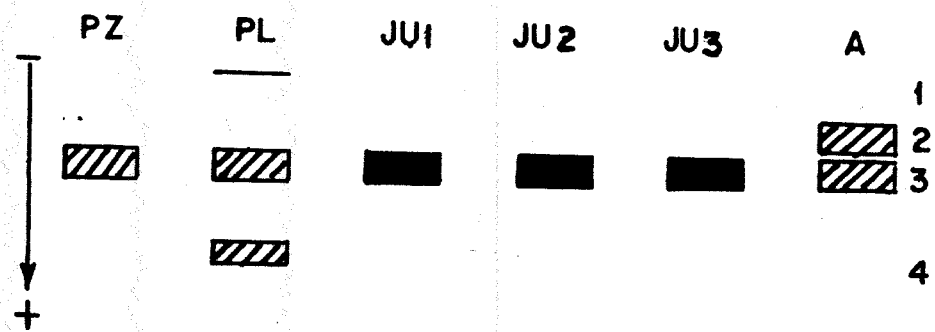
'-' represent the absence of the Isozyme

Table 35: Relative Mobility (RM) with intensity of Alcohol dehydrogenase bands separated in Penaeus indicus.

| Stage | Band Number | RM | Intensity |
|-------------------|-------------|-------------|-----------|
| Protozocea | 3 | 13.3 - 16.7 | x |
| Postlarva | 1 | 3.3 | x |
| | 3 | 13.3 - 16.7 | x |
| | 4 | 25.0 - 28.3 | x |
| 20-30mm(Juvenile) | 3 | 13.3 - 16.7 | xx |
| 40-50mm(") | 3 | 13.3 - 16.7 | xx |
| 70-80mm(") | 3 | 13.3 - 16.7 | xx |
| 90-120mm(Adult) | 2 | 8.3 - 11.7 | x |
| | 3 | 13.3 - 16.7 | x |

Fig. 8. Ontogenetic variation of alcohol dehydro-
genase enzyme in Penaeus indicus

Fig.8



PZ - Protozoa , PL - Post larva
 JU1 - 20 - 30 mm , JU2 - 40 - 50 mm
 JU3 - 70 - 80 mm , A - 90 - 120 mm

Table 36: Summary of Alcohol dehydrogenase patterns of Penaeus indicus based on Fig. 8.

| Stages | 1 | 2 | 3 | 4 | Total No. of bands |
|--------------------|---|---|----|---|--------------------------|
| Protozoa | - | - | + | - | 1 |
| Post larva | + | - | + | + | 3 |
| 20-30mm(Juveniles) | - | - | ++ | - | 1 |
| 40-50mm(") | - | - | ++ | - | 1 |
| 70-80mm(") | - | - | ++ | - | 1 |
| 90-120mm(Adult) | - | + | + | - | 2 |

'+' represents the presence of isozyme

'-' represents the absence of isozyme

Plate 6: Ontogenetic variation of acid phosphatase enzyme in Penaeus indicus a) Protozoa, b) Mysis, c) Post larva, d),e) juvenile, f) adult.

Plate 7: Ontogenetic variation of alcohol dehydrogenase enzyme in Penaeus indicus a) protozoa, b) post larva, c)-e) juveniles, f) adult

expressed 2 bands (Band No.1 & 2). Juvenile stages had 5 bands in Stage I, (Bands No.1, 2, 3, 5 & 6) 6 bands in Stage II, (Bands No.1, 2, 3, 5, 6 & 7), 4 bands in stage III, (Bands No.3, 5, 6 & 7) and 3 bands in Adult (bands No.3, 5 & 7). (Plate 8).

Bands No. 8 and 9 are found only in protozoa stage Band No.7 is expressed in juvenile II, and III and adult stages. Band No.6 is found in protozoa, juvenile and adult stages. Band No.5 is shown in mysis, in juvenile and adult stages. Band No.4 is seen in mysis stage only. Band No.3 is seen in protozoa, in juvenile and adult stages. Band Nos. 1 & 2 is found in post-larva and in juvenile stages I & II. Patterns obtained in juvenile and adult stages is only from hepatopancreas. Thus all the seven stages tested for aldehyde oxidase showed its stage specific patterns (Table No. 33 & 34).

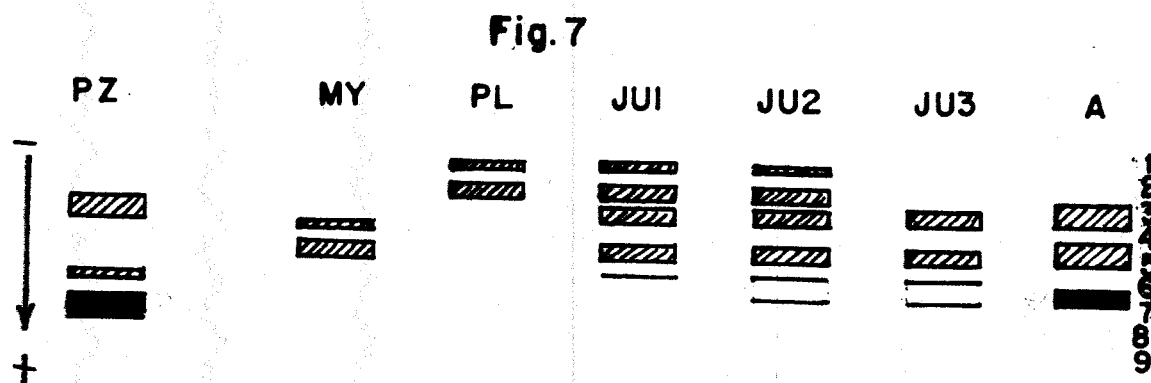
Alcohol dehydrogenase:

Alcohol dehydrogenase isoenzyme patterns were observed in Protozoa, Post-larva 3 different size groups of juveniles and adults (Fig. 8). Enzymatic activity of this particular enzyme was expressed by one band (Band No.3) in protozoa, 3 bands (Bands No.1, 3 & 4) in post-larva, one Band (Band No.3) in each stages of juveniles (Stage I-III) and 2 bands (Band No.2 & 3) in the adults stage (Plate 7).

Table 33: Relative Mobility with intensity of Aldehyde oxidase bands separated in Penaeus indicus.

| Stage | Band | RM | Intensity |
|--------------------|------|-------------|-----------|
| Protozocea | 3 | 16.7 - 20.0 | x |
| | 6 | 26.7 - 28.3 | x |
| | 8 | 30.0 - 31.7 | xxx |
| | 9 | 33.3 | x |
| Mysis | 4 | 20.0 - 21.7 | x |
| | 5 | 23.3 - 25.0 | x |
| Post-larva | 1 | 11.7 - 13.3 | x |
| | 2 | 15.0 - 16.7 | x |
| 20-30mm (Juvenile) | 1 | 11.7 - 13.3 | x |
| | 2 | 15 - 16.7 | x |
| | 3 | 18.3 - 20 | x |
| | 5 | 23.3 - 25 | x |
| | 6 | 26.7 | x |
| 40-50mm (Juvenile) | 1 | 11.7 - 13.3 | x |
| | 2 | 15.0 - 16.7 | x |
| | 3 | 18.3 - 20.0 | x |
| | 5 | 23.3 - 25.0 | x |
| | 6 | 26.7 | x |
| | 7 | 30.0 | x |
| 70-80mm (Juvenile) | 3 | 18.3 - 20.0 | x |
| | 5 | 23.3 - 25.0 | x |
| | 6 | 26.7 | x |
| | 7 | 30.0 | x |
| 90-120 mm(adult) | 3 | 17.3 - 20.0 | xxx |
| | 5 | 22.3 - 25.0 | xxx |
| | 7 | 28.0 - 30.0 | x |

**Fig. 7. Ontogenetic variation of aldehydeoxidase
enzyme in Penaeus indicus.**



PZ-Protozoa , MY-Mysis , PL-Post larva, JUI-20-30 mm
 JU2- 40- 50mm, JU3-70-80mm, A- 90-120mm

Table 34: Summary of Aldehyde oxidase patterns of Penaeus indicus based on Fig. 7.

| Stages | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Total |
|-------------------|---|---|---|---|---|---|---|------|---|-------|
| Protozoaea | - | - | + | - | - | + | - | (++) | + | 4 |
| Mysis | - | - | - | + | + | - | - | - | - | 2 |
| Post-larva | + | + | - | - | - | - | - | - | - | 2 |
| 20-30mm(Juvenile) | + | + | + | - | + | + | - | - | - | 5 |
| 40-50mm(") | + | + | + | - | + | + | + | - | - | 6 |
| 70-80mm(") | - | - | + | - | + | + | + | - | - | 4 |
| 90-120mm(Adult) | - | - | + | - | + | - | + | - | - | 3 |

'+' represents the presence of isozyme

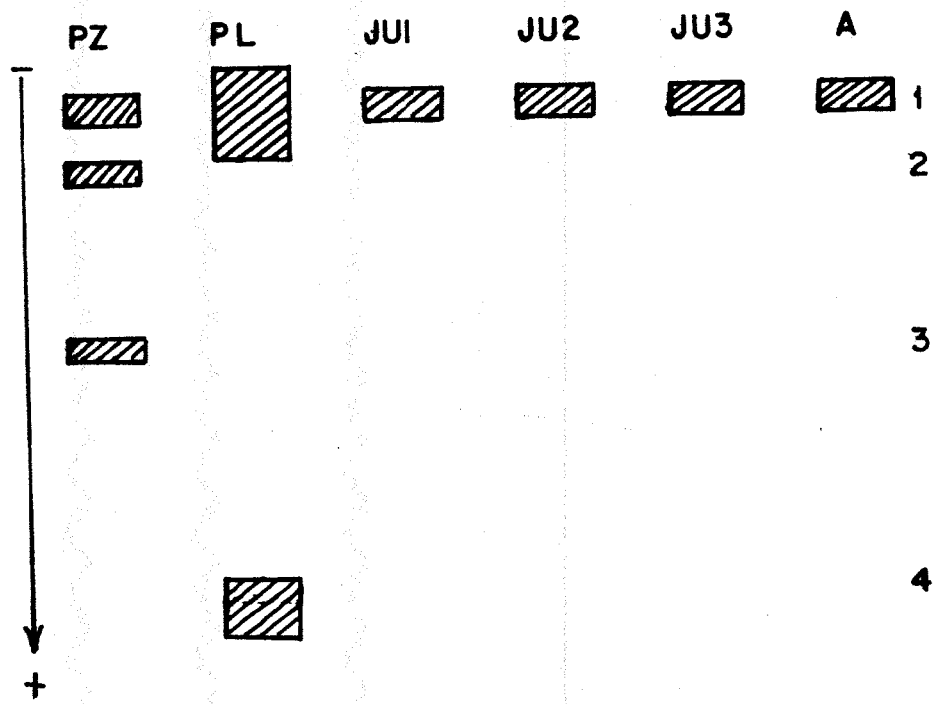
'-' represents the absence of isozyme

Table 37a: Relative mobility (RM) with intensity of
Esterase bands separated in *Penaeus indicus*.

| Stage | Band No. | RM | Intensity |
|---------------------|----------|-------------|-----------|
| Protozocea | 1 | 3.3 - 6.7 | x |
| | 2 | 11.7 - 15.0 | x |
| | 3 | 35.0 - 38.3 | x |
| Post larva | 1 | 0 - 11.7 | x |
| | 4 | 66.7 - 75 | x |
| 20-30mm (Juveniles) | 1 | 3.3 - 6.7 | x |
| 40-50mm (") | 1 | 3.3 - 6.7 | x |
| 70-80mm (") | 1 | 3.3 - 6.7 | x |
| 90-120mm(Adult) | 1 | 3.3 - 6.7 | x |

**Fig. 9. Ontogenetic variation of esterase
enzyme in Penaeus indicus.**

Fig. 9



PZ-Protozoa, PL-Post larva, JU1-20-30mm

JU2-40-50mm, JU3-70-80mm, A-90-120mm

Table 38: Summary of Esterase patterns of Penaeus indicus bands based on Fig. 9.

| Stages | Position of band | | | | Total No. of bands |
|-------------------|------------------|---|---|---|--------------------------|
| | 1 | 2 | 3 | 4 | |
| Protozoa | + | + | + | - | 3 |
| Postlarva | + | - | - | + | 2 |
| 20-30mm(Juvenile) | + | - | - | - | 1 |
| 40-50mm(") | + | - | - | - | 1 |
| 70-80mm(") | + | - | - | - | 1 |
| 90-120mm (Adult) | + | - | - | - | 1 |

'+' represent the presence of the Isozyme

'-' represent the absence of the Isozyme

Plate 8: Ontogenetic variation of Aldehyde oxidase enzyme in Penaeus indicus a) Protozoa, b) Mysis, c) Post larva d),e) juveniles, f) adult.

Plate 9: Ontogenetic variation of esterase enzyme in Penaeus indicus. a) Protozoa, b) Post larva c)-e) Juveniles, f) Adult

Band No.4 was found only in post-larva. Band No.3 was expressed in all the stages of development. Band No.2 was active only in the adult stage. Though post-larva and adult stages showed distinct stage specific pattern for the enzyme, juvenile stages, and Protozoa had identical single banded pattern (Band No.3 and Table No. 35 & 36).

Esterase:

Esterase isoenzyme separated in protozoa stage of P. indicus showed 3 bands (Band No.1, 2 & 3) whereas post-larval stage showed only 2 bands (Band No.1 & 4). In juvenile stages the enzyme separation was carried out in eye tissue alone and the isozyme activity was found to be exhibited only by one common band (Band No.1) in all three juvenile stages and adult (Table No.37a & 38). Hence, the esterase enzyme was showing specific pattern only in protozoa, post-larva juvenile and adult stages (Fig. 9 and Plate 9).

Malate dehydrogenase:

Malate dehydrogenase isoenzyme patterns were studied in detail only in post-larva, juvenile and the adult stages (Table No.37b). All stages expressed only one band with a

Table 37b: Relative mobility with intensity of malate dehydrogenase bands separated in Penaeus indicus.

| Stage | Band No. | RM Value | Intensity |
|--------------------|----------|-------------|-----------|
| Post larva | 1 | 16.7 - 23.3 | x |
| 20-30 mm(Juvenile) | 2 | 20 - 23.3 | x |
| 40-50 mm(") | 3 | 21.7 - 25 | x |
| 70-80mm (") | 4 | 23.3 - 26.7 | x |
| 90-120mm(Adult) | 4 | 23.3 - 26.7 | x |

Fig. 10. Ontogenetic variation of malate
dehydrogenase enzyme in Penaeus indicus.

Fig.10

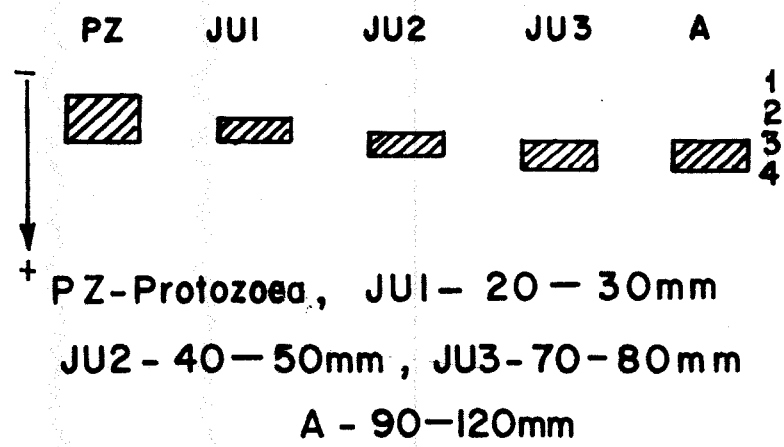
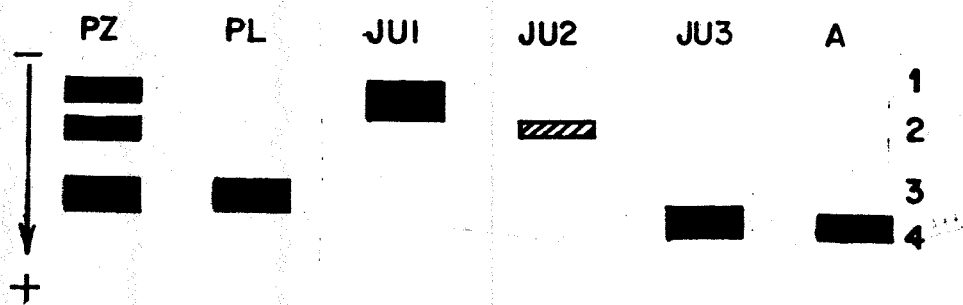


Table 39: Relative mobility (RM) with intensity of Octanol dehydrogenase bands separated in Penaeus indicus.

| Stage | Band No. | RM | Intensity |
|-------------------|----------|-------------|-----------|
| Protozoa | 1 | 0 - 3.3 | xx |
| | 2 | 5 - 8.3 | xx |
| | 3 | 13.3 - 16.7 | xx |
| Post-larvae | 3 | 13.3 - 16.7 | xx |
| 20-30mm(Juvenile) | 1 | 0 - 5 | xx |
| 40-50mm(") | 2 | 5 - 6.7 | x |
| 70-80mm(") | 4 | 16.7 - 20 | xx |
| 90-120mm(Adult) | 4 | 16.7 - 20 | xx |

**Fig. 11. Ontogenetic variation of octanol
dehydrogenase enzyme in Penaeus indicus.**

Fig. 11



PZ - Protozoa, PL - Postlarva, JU1 - 20 - 30mm

JU2 - 40 - 50mm, JU3 - 70 - 80mm, A - 90 - 120mm

Table 40: Summary of Octanol dehydrogenase patterns of Penaeus indicus based on Fig. 11.

| Stage | Position of Band | | | | Total No. of bands |
|-------------------|------------------|----|----|----|--------------------|
| | 1 | 2 | 3 | 4 | |
| Protozocea | ++ | ++ | ++ | - | 3 |
| Post larva | - | - | ++ | - | 1 |
| 20-30mm(juvenile) | ++ | - | - | - | 1 |
| 40-50mm(") | - | + | - | - | 1 |
| 70-80mm(") | - | - | - | ++ | 1 |
| 90-120mm(Adult) | - | - | - | ++ | 1 |

'+' represents the presence of the isozyme

'-' represents the absence of the isozyme

Plate 10: Ontogenetic variation of malate dehydro-
genase enzyme in Penaeus indicus
a) Protozoa, b), c) Juvenile, d) Adult

Plate 11: Ontogenetic variation of octanol dehydro-
genase enzyme in Penaeus indicus a) Protozoa,
b) Postlarva, c)-e) juvenile f) Adult.

slight variation in their relative mobility, width and intensity, indicating a lesser degree of stage specific pattern for the enzyme (Fig. 10 and Plate 10).

Octanol dehydrogenase:

Octanol dehydrogenase analysed in protozoa stage of P. indicus showed 3 bands (Band No.1, 2 & 3) whereas it showed only one band in post-larva (Band No.3), in all the juvenile (Band No.1 in stage I, band No.2 in stage II, Band No. in Stages III) and in adult stages (Band No.4) but the relative mobility, width and staining intensity varied. For all juvenile stages the eye tissue only was used for separation of this isozyme. Thus protozoa, post-larva and juvenile stages of 1, 2 & 3 and adult could be distinguished by this enzyme pattern (Table No. 39 & 40 and Plate 11).

General Protein:

Protein patterns analysed in muscle tissue of P. indicus showed an enhancement in the number of fractions as the development progresses from juvenile to adult stages giving 12 bands in stage I (20-30 mm) 14 bands in stage II (40-50 mm) 15 bands in Stage III (70-80 mm) and 17 bands in adult stage (90-120 mm) (Fig. 12 and Plate 12).

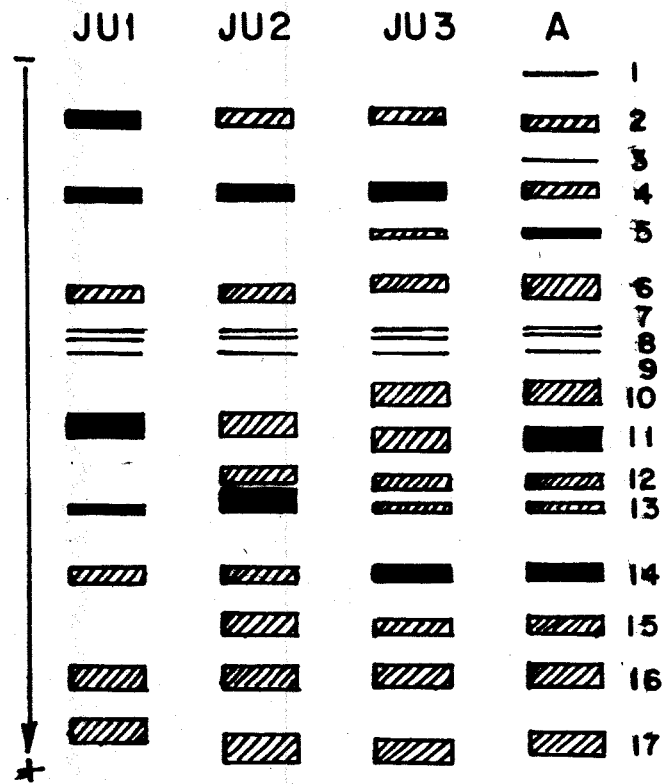
Table 41: Relative mobility (RM) with intensity of muscle myogen patterns of Penaeus indicus

| Band No. | RM | Intensity |
|--------------------|-------------|-----------|
| 20-30mm (Juvenile) | | |
| 1 | 0 | x |
| 2 | 4.3 - 5.7 | xx |
| 3 | 14.2 - 15.7 | xx |
| 4 | 27.1 - 28.6 | x |
| 5 | 32.9 | x |
| 6 | 34.3 | x |
| 7 | 35.7 | x |
| 8 | 44.3 - 47.1 | xx |
| 9 | 55.7 - 57.1 | xx |
| 10 | 64.3 - 65.7 | x |
| 11 | 77.1 - 80 | x |
| 12 | 84.2 - 85.7 | x |
| 50-60mm (Juvenile) | | |
| 1 | 0 | x |
| 2 | 4.3 - 5.7 | x |
| 3 | 14.2 - 15.7 | xx |
| 4 | 27.1 - 28.6 | x |
| 5 | 32.9 | x |
| 6 | 34.3 | x |
| 7 | 35.7 | x |
| 8 | 44.3 - 47.1 | x |
| 9 | 51.4 - 52.9 | x |
| 10 | 54.3 - 57.1 | xx |
| 11 | 64.3 - 65.7 | x |
| 12 | 70.0 - 72.9 | x |
| 13 | 77.1 - 80.0 | x |
| 14 | 85.7 - 90.0 | x |

| Band No. | RM | Intensity |
|---------------------|-------------|-----------|
| 70-80 mm (Juvenile) | | |
| 1 | 0 | x |
| 2 | 4.3 - 5.7 | xx |
| 3 | 14.2 - 15.7 | x |
| 4 | 20.0 - 21.4 | x |
| 5 | 25.7 - 27.1 | x |
| 6 | 32.9 | x |
| 7 | 34.3 | x |
| 8 | 35.7 | x |
| 9 | 40.0 - 42.9 | x |
| 10 | 45.7 - 48.8 | x |
| 11 | 52.9 - 54.3 | x |
| 12 | 55.7 - 57.1 | x |
| 13 | 64.3 - 65.7 | xx |
| 14 | 71.4 - 72.9 | x |
| 15 | 77.1 - 80.0 | x |
| 16 | 87.1 - 90.0 | |
| 90-120 mm (Adult) | | |
| 1 | 0 | x |
| 2 | 5.7 - 7.1 | x |
| 3 | 11.4 | x |
| 4 | 14.2 - 15.7 | x |
| 5 | 20.0 - 21.4 | xx |
| 6 | 25.7 - 28.6 | x |
| 7 | 32.9 | x |
| 8 | 34.3 | x |
| 9 | 35.7 | x |
| 10 | 40.0 - 42.9 | x |
| 11 | 45.7 - 48.8 | xx |
| 12 | 52.0 - 54.3 | x |
| 13 | 55.7 - 57.0 | xx |
| 14 | 64.3 - 65.7 | xx |
| 15 | 71.4 - 72.9 | x |
| 16 | 77.1 - 80.0 | x |
| 17 | 85.7 - 88.6 | x |

**Fig. 12. Ontogenetic variation of general
protein in Panæus indicus.**

Fig. 12



JU1 — 20 — 30 mm

JU2 — 40 — 50 mm

JU3 — 70 — 80 mm

A — 90 — 120 mm

ble 42: Summary of muscle myogen pattern of General protein based on their ontogeny Fig. 12.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | Total No.of bands | Com- mon bands | No.of dele- ted bands | Tot- al | No.of addit- ional bands |
|--------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|-------------------------|----------------------|--------------------------------|------------|-----------------------------------|
| <u>indicus</u> | | | | | | | | | | | | | | | | | | | | | | |
| 2-30 mm(Juveniles) | + | + | - | + | - | + | + | + | + | - | + | - | + | + | - | + | + | 12 | 12 | 5 | | 0 |
| 2-50 mm (") | + | + | - | + | - | + | + | + | + | - | + | + | + | + | + | + | + | 14 | 12 | 3 | | 1 |
| 2-80 mm (") | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 16 | 12 | 1 | | 3 |
| 2-120mm (Adult) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 17 | 12 | 0 | | 5 |

'+' represents the presence of the protein band

'-' represents the absence of the pretein band

When the analysis was done according to the relative mobility of these bands, the common bands seen in all stages, the total deleted bands and the exact number representing their location can be compared. Thus 12 bands are seen representing their common nature in all stages. Stage I is found to lack 5 numbers of bands viz. band No. 3, 5, 10, 12 & 15, stage II lacks band No.3, 5, & 10 and stage III lacks band No.3. Though there are more number of common bands, absence or presence of a few additional bands again indicated specific general protein pattern for the different stages tested here. (Table No. 41 & 42).

Discussion:

Due to commercial importance, studies on Larval development of penaeid prawns gained much importance in the last 3 decades. Rearing of penaeid shrimp from eggs to post-larvae and their culture practice is explained in detail by Cook and Murphy (1966, 1969, 1971). Information regarding developmental stages of various prawns like Penaeid duorarum (Dobkin, 1961), Penaeus setiferus (Heegaard 1953), Penaeus japonicus (Hudinaga 1935), Metapenaeus joyneri (Lee and Lee 1968, 1969), Metapenaeus dobsoni (Menon 1951), commercially important Penaeus sp. of prawns (Williams 1953, 1955, 1959), Metapenaeus sp. of prawns (Muriel and Bennett 1951) and penaeid prawns of south west coast of India (Mohamed, Rao and George 1967) are available. Rao (1973) had explained clearly the different stages of Parapenaeopsis stylifera, Metapenaeus monoceros, M. affinis, M. dobsoni and Penaeus indicus. Paulinose (1982) has given the key for the identification of larvae and postlarvae of the penaeid prawns. Artificial hatchery production and rearing of penaeid prawn seed of P. indicus is attempted in many parts of the country and the techniques adopted for this at Narakkal Prawn Culture Laboratory CMFRI is given in CMFRI (1985). Some of the morphological

differences between the same stages of different species are very minute, making it often difficult to identify them in the field. So an alternate method for identifying would be advantageous.

Genetic codes in DNA are translated into structural proteins of each organism. Some of these proteins exist in the form of isoenzymes. Electrophoretic separation of isoenzymes such as acid phosphatase, aldehyde oxidase, alcohol dehydrogenase, malate dehydrogenase, octanol dehydrogenase, esterase, and general protein were carried out in different developmental stages in Indian White Prawn Penaeus indicus.

Cellular components increases during development due to differential synthesis of isoenzymes bringing out certain natural changes in the quantity and quality of each enzyme in each stage of development. Therefore, the isoenzymes which are specific gene products can be identified and used as efficient genetic markers at a particular developmental stage. Biochemical identification of each stage will give their extent of, differentiation, relationship and closeness of different stages of development. Changes observed in different developmental stages are measured quantitatively and qualitatively by separating different enzymes using electrophoresis.

Acid phosphatase:

Ontogenetic changes in Acid phosphatase in different developmental stages like young larva, 3rd instar larvae, pupa, of Drosophila melanogaster showed faster mobility as it reached adult stage with only one band in each stage (Beckman and Johnson 1964) whereas in Xanthid crab Morgan et al, (1978) found 2 bands in Stage III & IV, one common band and one with varying mobility in zoea stage, 3 bands in megalopa stage with varying mobility and 2 bands in young crab with same mobility. In the fish Liza parsia, Mary (1985) found 3 bands in the eye tissues of fingerlings and 4 bands (2 bands showing different mobility) in the eye tissues of adult. Thus different organisms indicated ontogenetic changes in the expression of acid phosphatase isoenzymes.

Similarly, Lester and Cook (1987) reported definite ontological changes in the electrophoretic patterns of acid phosphatase in four species of Penaeus. The ontological patterns of the enzyme in each of these species indicate an inter species ontological patterns for each larval stage besides ontological changes in each species. The different developmental stages of P. indicus studied here for the first time also showed distinct ontological patterns for the acid phosphatase (Fig. 6).

Thus protozoa stage showed 2 bands in P. aztecus, one band in P. setiferus, 3 bands in P. stylirostris 2 bands in P. vannamei (Lester and Cook 1987) and 4 bands in P. indicus. Mysis stage expressed 3 bands in P. aztecus, 2 bands in P. setiferus, 2 bands in P. stylirostris 2 bands P. vannamei (Lester and Cook 1987) but in the present study 3 bands in P. indicus. The relative mobility showed difference from P. aztecus which alone has equal number of bands as P. indicus. Post larval stage showed one band in P. aztecus, 2 bands in P. setiferus, 3 bands in P. stylirostris and one band in P. vannamei (Lester and Cook, 1987) and expressed 3 bands in P. indicus. But here again the relative mobility showed variation from P. stylirostris which has 3 bands as in P. indicus.

P. indicus showed 4 bands in protozoa, 3 bands each in mysis and post-larva, 3, 2, 2 and 1 bands in three different stages of juvenile and adult stages respectively indicating stage-specific acid phosphatase patterns either in terms of the number of bands, electrophoretic mobility of different bands or even width and intensity of certain bands.

Though mysis, post larva and juvenile stage I of P. indicus had three bands, each of these stages could

still be separated on the basis of difference in electrophoretic mobility pattern of the enzyme. A comparison of Acid phosphatase patterns observed by Lester and Cook (1987) and the present observations shows that even each larval stage of different species can be clearly identified on the basis of species specific number of bands or where similar number of bands may exist difference in the electrophoretic mobility of the bands.

Aldehyde oxidase:

Ontological changes in the Aldehyde oxidase of P. indicus described for the first time here also showed a developmental stage specific pattern particularly in the expression of number of bands as different larval stages progress from protozoa to adult.

Aldehyde oxidase in P. indicus studied here also showed a pattern different from that of Penaeus setiferus, P. stylirostris, P. aztecus and P. vannamei of Lester and Cook (1987). Protozoa stages of P. aztecus, P. setiferus, P. stylirostris, and P. vannamei showed 1, 4, 2 and 1 band respectively whereas P. indicus expressed 4 bands. Mysis stage of P. aztecus, P. setiferus, P. stylirostris and P. vannamei showed 2, 2, 4 and 1 bands whereas P. indicus

was found to have 2 bands. Post larval stage of P. aztecus, P. setiferus, P. stylirostris and P. vannamei had 2, 3, 1 and 1 bands. P. indicus also showed 2 bands. In the adult pattern P. aztecus, P. setiferus, P. stylirostris, and P. vannamei expressed 1, 2, 3 and 2 bands whereas P. indicus has 3 bands (Table 34).

Therefore, aldehyde oxidase detected and described here for the first time in P. indicus possess Ontological as well as species specific patterns as also indicated by the pattern of acid phosphatase discussed earlier.

Alcohol dehydrogenase:

Ontogeny changes of Alcohol dehydrogenase observed in leopard danio showed activity in liver cells (Frankel 1981) and this was correlated with the changes over in metabolic pathway to liver due to depletion of yolk in the later stage of development (Shaklee et al, 1974).

Present investigation carried out in the different stages of P. indicus showed for the first time the stage specific patterns of alcohol dehydrogenase. In the protozoa and first three juvenile stages it expressed a single zone of enzyme activity whereas post-larval stage expressed 3 bands which are distinct and the adult stage showed only 2

bands. As already explained post larva stage showed maximum of 3 bands whereas all other stages except the adult stage showed only one band. Though band No.3 is common to all stages specific pattern of alcohol dehydrogenase can also be used for separating different larval stages of P. indicus.

Esterase

Ontogenetic study of esterase patterns of various cavian tissues showed marked changes to occur in the liver, Kidney and intestine (Holmes and Masters 1967). Hunter et al, (1964) described changes occurring in the development of liver and kidney of the foetal and weaning mouse. Paul and Fottrell (1961) found foetal human tissue esterase isoenzyme pattern to resemble those of adult but Blanco and Zinkhan (1966) reported an increase in both the number of isoenzymes and their activities during development.

Various workers like Gooch (1977), Morgan et al, (1980) and Kannupandi (1980) have investigated on the ontological changes of esterase in the different stages of crabs. Morgan et al, (1980) found 2, 3, 3 and 7 bands during the zoeal stages I-IV, 5 bands in megalopa stage and 4 bands in young crab stage. Kannupandi (1980) found out a gradual increase in number of esterase bands upto Zoea stage IV and thereafter the zone of activity decreased in the later stages.

Lester and Cook (1987) reported ontological changes in esterase in the different stages such as protozoa, mysis, postlarva and adult of Penaeus species of prawns like Penaeus aztecus, P. setiferus, P. stylirostris and P. vannamei. Similarly, the esterase patterns in P. indicus studied here also showed characteristic bands for each larval stages (Fig. 9).

When the esterase of different larval stages of P. indicus detected here are compared with that of other species of Penaeus as reported by Lester and Cook (1987), definite species specific esterase patterns are indicated in different larval stages of each species Protozoa of P. aztecus, P. setiferus, P. stylirostris and P. vannamei showed 4, 5, 4 and 3 bands respectively (Lester and Cook 1987) whereas P. indicus expressed 3 bands.

Postlarva stage expressed 9, 8, 9 and 1 bands respectively in P. aztecus, P. setiferus, P. stylirostris and P. vannamei whereas P. indicus expressed only 2 bands, Esterase showed only one band in all the juvenile and adult stages of P. indicus probably due to the analysis of this enzyme in Eye tissue.

Hence ontological changes in the esterase of P. indicus studied here are also comparable with that of other species as well as with that of other enzymes discussed earlier.

Malate dehydrogenase:

Ontological changes were observed in malate dehydrogenase enzyme in crabs by Gooch (1977) and Morgan et al, (1978) and in plants by Rider (1980). Morgan et al, (1978) showed an increase in number of bands as the animal becomes adult. In P. indicus the expression of malate dehydrogenase enzyme was studied in Postlarva, three different stages of juveniles and adult. Though the malate dehydrogenase consisted of only a single band in all the stages slight variation in the relative mobility of the single band indicated its own stage specific nature of the enzyme as described in other enzymes but to a lesser degree.

Octanol dehydrogenase:

The octanol dehydrogenase enzyme in P. indicus tested in the present study also exhibited stage specific pattern of the enzyme.

Protozoa with 3 bands, could be easily distinguished from postlarva, all juvenile and adult stages with a single band. Though postlarva juvenile and adult stages had only

one band, differences in the relative mobility and staining intensity showed expected ontological changes (Fig. 11). Band No.1 present in protozoa is present in juvenile stage I and the Band No.2 present in protozoa is also present in juvenile stage 2 whereas the band No.3 found in protozoa and postlarvae is absent in all juvenile stages and the band No.4 which is present in juvenile stage 3 and adult is found to be absent in all other stages.

Thus ontological changes in the acid Octanol dehydrogenase showed a specific pattern particularly in the number of bands between protozoa and other larval stages.

General protein:

Ontological studies of general protein in salmon showed changes with gradual transition of the protein pattern showing an increase in number from newly hatched having 9 bands to 12 bands in sexually matured salmon (Nyman 1967). Bhattacharya and Alfred (1982) observed an increase in quantity of protein in the tissues of brain and muscle and a decrease in protein content of liver with the increase in size of Channa stewartii and Danio dangila. Sriraman and Reddy (1977) found out 9 bands in the size group 13-15 mm, 10 bands in size group 18-20mm and 10 & 11 bands in the adult stage of P. indicus and P. monodon, Whereas Prathibha

(1984) found out 11, 14 and 16 bands in 10-15 mm post-larva, 30-70 mm juvenile and 70-190 mm adult.

Lester and Cook (1987) got 1, 2, 1, 0 bands in protozoa, 3, 2, 4 & 4 bands in Mysis, 8, 8, 6 & 5 bands in postlarvae and 11, 8, 10 & 12 bands in adult of P. aztecus, P. setiferus, P. stylirostris and P. vannamei respectively. In the present study 11, 12, 14, 16 & 17 bands were obtained in juveniles of size groups of 20-30 mm, 40-50 mm, 70-80 mm and Adult 90-120 mm in P. indicus (Table 43).

Penaeus indicus studied here showed an L-III increase in the number of fractions from juveniles to adult showing 12, 14, 16 and 17 numbers respectively. This type of striking increase in band diversity alongwith development is also noticed in other Penaeus spp. (Lester and Cook 1987) and also in Xanthid crab by Kannupandi (1980).

Though 12 out of 17 general protein bands in all the larval stages of P. indicus studied here were similar in electrophoretic mobility, absence or presence of a few additional bands, again, clearly separated different larval stages.

The separation of different isoenzymes like, acid Phosphatase, alcohol dehydrogenase, aldehydeoxidase, esterase,

Table 43: Total number of Muscle myogen protein patterns found in different penaeid prawns.

| | | | | Total |
|------------------------|-------------------|-------|------------|-------|
| Sriraman & Reddy 1977* | | | | |
| <u>P. indicus</u> | 13-15 mm | | | 9 |
| | 18-20 mm | | | 10 |
| | Adult | | | 10 |
| <u>P. monodon</u> | 13-15 mm | | | 9 |
| | 18-20 mm | | | 10 |
| | Adult | | | 11 |
| Prathibha 1984** | | | | |
| <u>P. monodon</u> | 10-15 PL mm | | | 12 |
| | Juvenile 30-70 mm | | | 14 |
| | Adult 70-190 mm | | | 16 |
| Lester & Cook 1987*** | | | | |
| | Protozoa | Mysis | Postlarvae | Adult |
| <u>P. aztecus</u> | 1 | 3 | 8 | 11 |
| <u>P. setiferus</u> | 2 | 2 | 8 | 8 |
| <u>P. stylirostris</u> | 1 | 4 | 6 | 10 |
| <u>P. vannamei</u> | - | 4 | 5 | 12 |
| Present study | | | | |
| <u>P. indicus</u> | 20-30 mm | | | 12 |
| | 40-50 mm | | | 14 |
| | 70-90 mm | | | 16 |
| | Adult | | | 17 |

protein, octanol dehydrogenase and malate dehydrogenase in different developmental stages of the prawn P. indicus studied here revealed stage and species specific characteristic bands as seen in Xanthid crab (Kannupandi 1980) and in P. monodon (Prathibha 1984). Lester (1980) used these patterns for identification of juveniles of different species of prawns and Morgan et al, (1980), for the identification of different stages of crab. Lester and Cook (1987) expressed abundant changes in isozyme pattern among different development stages of penaeid prawn. Hedgecock et al, (1982) stated that there is constancy of Zymogram pattern throughout the development of decapods species and Gooch (1977) found no change in enzyme expression of life cycle of crabs. This is in contradiction with the present results as well as some of the earlier reports, suggesting that ontological changes are either not essential for all species or not sufficiently expressed as to detect it biochemically.

On comparison of the total number of bands possessed by protozoa and adult stages of P. indicus studied here it is interesting to note that ontological changes in the acid phosphatase reduced the three banded pattern of protozoa to a single banded pattern in the adult, whereas the number of bands were found increased in P. setiferus, P. stylirostris and P. vannamei while retaining the same number of P. aztecus.

Therefore the ontological reduction in the total number of acid phosphatase enzyme bands as noticed in the present study of P. indicus is not a general phenomenon of ontological changes. Such ontological shift in the total number of bands and even electrophoretic mobility of certain bands is also noticeable to some extent in other enzymes of P. indicus. The species specific and stage specific biochemical complexity of each species alone could be accounted for the above phenomenon.

The protein present in initial stage of development migrate to a different position due to changes in the cytoplasmic milieu or post-translational modification as found in Penaeus sp. of prawns (Lester and Cook 1987) and thus ontogenetic modification of gene action leading to increased differentiation of tissues and organs with increasing age of the organism (Morgan et al, 1973).

Increased isozyme complexity corresponds to enhancement in dietary requirement (Frank et al, 1975) and also changes occur for metabolic preparation for the metamorphic period (Costlow 1968, Oconnor and Gilbert 1968, Yamoka and Scheer 1970).

Characteristic changes observed in isozyme patterns may thus reflect changes in cell components and synthesis of

isoenzyme in relation to gene expression. This may also be due to essential physiological adaptive changes brought about during habit shift from marine to brackish water as reported in crabs when changing from Pelagic to benthic environments (Morgan et al, 1978 and Kannupandi 1980).

To conclude the distinct ontological patterns of different isoenzymes and general proteins discovered and described here should enable one to easily identify different larval stages of P. indicus from that of other prawn species.

CHAPTER VI

INTRASPECIES ENZYME LOCI AND THEIR VARIATION

Resume of literature:

Biochemical genetics is an effective tool in detecting single gene variation and this paved way to resolve several problems faced in fish population studies.

It aims at establishment of the pattern of population structure of the species, analysis of genetic differences between populations, demarkating boundaries between adjacent populations to show the extent of isolation, for the study of evolutionary processes in populations, possible side effects due to modern techniques on population structure, and to protect the natural resources of the commercially important fishes (Awise et al., 1975, Kirpichnikov 1981). Loss of genetic variation found in the artificially cultured population can be traced out and possible genetic manipulation can be recommended (Allendorf et al., 1979). Genetics is being employed in aquaculture to select diverse parental stocks and to produce progeny which show hybrid vigour (Hedgecock et al., 1976). Genetic differentiation without isolation in the American eel was found out (Williams et al., 1973, Koehn and Williams 1978). Biochemical variants were

used in Pacific salmon and Rainbow trout among populations in identification and characterization of population (Utter et al., 1973). Geographic patterns of Zoarces viviparus was identified by Christiansen et al. (1974). Population analysis on German trout was done by electrophoresis (Keese and Langholz 1974). May et al., (1975) examined inter and intra specific genetic variation in pink salmon (Oncorhynchus gorbuscha) and Chum Salmon (Oncorhynchus keta). Avise et al., (1975) found out the genetic change due to adaptive differentiation between two native California minnows. Genetic variation studies in Scandinavian brown trout (Salmo trutta L.) showed sympatric populations (Allendorf et al., 1976) Christiansen and Simonsen (1978) found out the geographic variation in protein polymorphism in the eelpout Zoarces Viviparus (L). Rodino and Comparini (1978) found out the genetic variability in the European eel Anguilla anguilla L. Ward (1977) worked on the protein variation in plaice Pleuronectes platessa L. Mangaly & Jamieson (1978) used three genetic tags to find out the unit stock nature of the European hake, Merluccius merluccius (L). Koehn and Williams (1978) traced the genetic difference in the American eel Anguilla rostrata without any isolation. Enzyme polymorphisms in the Atlantic Mackerel, Scomber Scombrus L. was found out by Smith and Jamieson (1978; 1980). Genetic variation and

population structure of New Zealand snapper Chrysophrys auratus (Forster) was found out by Smith et al., (1978).

Awise and Felley (1979) showed the population structure of freshwater fish Bluegill (Lepomis macrochirus). Kirpicknick and Selander (1979) traced the supporting evidence of speciation in lake white fish (Coregonus culpeaformis). Protein variation studies revealed subspeciation in cutthroat trout Salmo clarki (Loudenslager and Gall, 1980). Population structure of lake white fish Coregonus clupeiformis was analysed using 4 populations by Imhof et al., (1980). Wishard et al., (1980) worked on biochemical genetic characteristics of native trout population of owyhee country, Idaho. Gary (1980) showed geographic variation in milk fish Chanos chanos with biochemical evidence using 38 loci from 14 locations. Electrophoretic variation studies in four strains of rainbow trout (Salmo gairdneri Richardson) was done by Guyomard (1981). Ecological, morphological and electrophoretic variation among allopatric Ontario lake white fish (Coregonus clupeaformis) stock was studied by Ihssen et al., (1981). Population structure of spanish mackerel Scomberomorus maculatus was found out by Johnson (1981).

The existence of taxonomically distinct strains of Brook trout (Salvelinus fontinalis) was investigated by

Mark et al., (1981). Genetic distinctness of large mouth bass (Micropterus salmoides) populations from different geographic regions were studied by Philip et al., (1981). Origin of Rainbow trout population was traced by exposing them in different ecological niches (Reichle 1981). Gene frequency difference of New Zealand ~~loki~~ Macruronus novaezelandiae from different locations were analysed (Smith et al., 1981). Krieg and Guymard 1984 gave an account of the genetic differentiation between brown trout populations. Electrophoresis analysis of Australian barramundi suggested the existence of multiple stocks (Shaklee and Salini, 1983). Biochemical genetics of Atlantic herring (Clupea harengus) and Pacific herring (Clupea Pallasii) was studied by Grant and Utter (1984) and Grant (1984). Milner et al., (1985) identified the naturally occurring mixed population of Pacific salmon Oncorhynchus sp. Electrophoretic assessment of stocks of brown trout Salmo trutta L. suggested a supplemental stocking programme (Taggart & Ferguson 1986). Biochemical genetic variation in angler fish Lophius piscatorius was done by Crozier (1987).

A review of the literature available on electrophoretically detectable genetic variations in natural populations shows considerable work, on this line on several isoenzymes in crustaceans. Details of these studies are summarised in the table given below:-

Electrophoretically Detectable Genetic Variation in Natural Populations of Crustaceans^a

| Subphylum | Class | Order | Species ^b | Group 1 | | | | | | | | | | | | | | | | |
|------------------------------------|----------|-------|----------------------|----------|----------|-----|-----|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----|----------|
| | | | | ALDO | FUM | GDH | GOT | G-3PDH | α-GPDH | HK | IDH | LDH | MDH | ME | MPI | PGM | 6-PGDH | PGI | TPI | YDH |
| Branchiopoda | | | | | | | | | | | | | | | | | | | | |
| Diplostraca | | | | | | | | | | | | | | | | | | | | |
| Conchostraca | | | | | | | | | | | | | | | | | | | | |
| Daphnia | | | | | | | | | | | | | | | | | | | | |
| magna(1) | | | | | | | | | | | 2M | | .53 3 | | | | | | | 1M |
| Daphnia | | | | | | | | | | | | | | | | | | | | |
| pulex(2) | | | | 1M | | | 1M | | | | | | 2M | | | | 1M | | 1M | |
| Simocephalus | | | | | | | | | | | | | .53 | | | | | | .06 | |
| serrulatus | | | | | | | | | | | | | | | | | | | | |
| (3) | | | | | | | | | | | | | 3 | | | | | | 2 | |
| Maxillopoda | | | | | | | | | | | | | | | | | | | | |
| Copepoda | | | | | | | | | | | | | | | | | | | | |
| Harpacticoida | | | | | | | | | | | | | | | | | | | | |
| Tisbe holothyrinae(4) | | | | | | | | | | | | | .14 2 | .29 3 | | .42 3 | | .39 3 | | .23 3 |
| Cirripedia | | | | | | | | | | | | | | | | | | | | |
| Thoracica | | | | | | | | | | | | | | | | | | | | |
| Chthamalus | | | | | | | | | | | | | | | | | | | | |
| stellatus (Poli)(5) | .45 3 | | | | | | | 1M | | | | | .05 2 | | .13 5 | .09 4 | | .05 4 | | |
| Chthamalus | 1M | | | | | | | 1M | | | | | .32 2 | | .15 3 | .46 8 | | .27 7 | | |
| montagu(5) | | | | | | | | | | | | | | | | | | | | |
| Chthamalus | | | | | | | | | | | | | | | | | | | | |
| dalli Pilsbry(6) | 1M | | | | | | | 1M | | | .33 2 | 1M | .07 3 | 3M | | .48 3 | .33 2 | .46 3 | | .08 2 |
| Chthamalus | | | | | | | | | | | | | | | | | | | | |
| fissus Darwin(6) | .03 2 | 1M | | .07 3 | | | 1M | | .02 2 | .16 3 | | .05 2 | | .06 3 | 1M | | .33 3 | .51 4 | | 1M |
| Chthamalus | | | | | | | | | | | | | | | | | | | | |
| anisopoma Pilsbry(6) | .10 2 | 1M | | .12 2 | | | | | .38 2 | 1M | | .10 2 | | .05 2 | .14 2 | | .35 3 | .50 3 | | |
| Chthamalus | 1 M | 1M | | .14 | | | 1M | | .02 2 | 1M | | .04 2 | | .15 3 | | | .36 3 | 1M | | .14 2 |
| sp.(6) | | | | | | | | | | | | | | | | | | | | |
| Chthamalus | | | | | | | | | | | | | | | | | | | | |
| sp.(6) | .28 3 | 1M | | 2M | .08 2 | | | | .02 2 | 1M | | .04 2 | | .21 3 | 1M | 1M | | .40 3 | | 1M |
| Balanus | | | | | | | | | | | | | | | | | | | | |
| amphitrite Darwin(7) | 1M | | | .37 3 | .11 2 | | | | | 1M | | .05 2 | | .31 3 | .52 3 | | | .14 3 | | |
| Balanus | | | | | | | | | | | | | | | | | | | | |
| amphitrite inexpectatus Pilsbry(7) | 1M | | | .11 2 | 1M | | | | 1M | | | .03 2 | | .54 4 | .08 3 | | | .43 3 | | |
| Balanus | | | | | | | | | | | | | | | | | | | | |
| glandula Darwin(7) | .18 2 | | | .50 2 | 1M | | | | | .06 2 | 1M | .08 3 | | .75 6 | .51 4 | | | .64 6 | | |
| Balanus | | | | | | | | | | | | | | | | | | | | |
| crenatus Brugliere(7) | 1M | | | .36 3 | .1M | | | | | 1M | | 2M | 1M | .70 4 | .09 3 | | | .60 4 | | |
| Semibalanus | | | | | | | | | | | | | | | | | | | | |
| cariculus (Pallas)(7) | 1M | | | .36 2 | | | | | | | | .06 3 | 1M | .64 5 | | | | .14 4 | | |
| Hoplocarida | | | | | | | | | | | | | | | | | | | | |
| Hoplocarida | | | | | | | | | | | | | | | | | | | | |
| Stomatopoda | | | | | | | | | | | | | | | | | | | | |
| Squilla nepa(8) | .10 2 | 1M | | 2M | 1M | | | | 1M | 1M | | 2M | 1M | .03 2 | 1M | | .56 3 | .38 2 | 1M | .16 2 |
| Squilla | | | | | | | | | | | | | | | | | | | | |
| woodmasoni (8) | | | | 1M | | | | | | | | | 1M | 1M | .25 2 | | | 1M | 1M | |

Contd...

| Phylum | | | Group 1 | | | | | | | | | | | | | | | | | |
|--------------|--------------|---------------------------------------|----------|----------|-----|----------|--------|---------|----------|----------|-----------|-----------|----------|----------|----------|----------|----------|----------|-----|--|
| Class | Order | Species ^b | ALDO | FUM | GDH | GOT | G-3PDH | G-6PDH | HK | IDH | LDH | MDH | ME | MPI | PGM | 6-PGDH | PG1 | TPI | XDH | |
| Malacostraca | | | | | | | | | | | | | | | | | | | | |
| | Peracarida | | | | | | | | | | | | | | | | | | | |
| | | Isopoda | | 1M | | | 1M | | 1M | .41 2 | 1M | .22 2 | 1M | 1M | | 1M | .39 | 1M | | |
| | | Exciridiana caidii(7) | | | | | | | | | | | | | | | | | | |
| | | Exciridiana Sp.(7) | | 1M | 1M | | 1M | | 1M | 1M | | 2M | 1M | .44 3 | 1M | 1M | .54 3 | 1M | | |
| | Eucarida | | | | | | | | | | | | | | | | | | | |
| | | Euphausiacea | | | | | | | | | | | | | | | | | | |
| | | Euphausia superba(9) | .28 2 | 2M | | .49 3 | 1M | 2M | .06 3 | 1M | | .22 3 | 2M | | 1M | 1M | .35 4 | 1M | 1M | |
| | | Euphausia micronata(10) | .32 3 | .25 4 | | .11 3 | 1M | 1M | .40 3 | | .22 4 | .13 2 | .32 4 | .37 4 | | .11 3 | .20 6 | .06 3 | | |
| | | Euphausia distingenda (10) | .46 4 | .26 3 | | .13 5 | 1M | 1M | .55 4 | | .20 3 | .41 3 | .20 5 | | .42 6 | .19 4 | .33 6 | .13 4 | | |
| | Decapoda | | | | | | | | | | | | | | | | | | | |
| | | Penaeidae | | | | | | | | | | | | | | | | | | |
| | | Penaeus merguensis de Man(8) | 1M | | 1M | 1M | 1M | 1M | | | 1M | 2M | 1M | .11 5 | .03 3 | 1M | .04 4 | 1M | | |
| | | Penaeus aztecus Ives (11) | 1M | | | 1M | 1M | | .28 3 | | | .03 3 | | .40 6 | .33 4 | .06 3 | .41 4 | | | |
| | | Penaeus duorarum Burkenroad(11) | | | 1M | 1M | 1M | | .02 3 | 1M | | .26 3 | | .31 7 | .09 4 | .45 3 | .10 3 | | | |
| | | Penaeus setiferus Linnaeus(11) | | | 1M | | 1M | | .17 3 | 1M | | 1M | | .37 6 | .33 5 | .18 3 | .26 5 | | | |
| | | Penaeus stylirostris (23) | | | | | | | | | | 1M | | | | 1P | | | | |
| | | Penaeus Vannamei (23) | | | | | | | | | | 1M | | | | 1P 3 | | | | |
| | | Metapenaeus macleayi(25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | M.bennett- ae(25) | | | | | | 1M | | | | 1P | | | | 1M | | | | |
| | | M.endeavou- ri(25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | M.ensis(25) | | | | | | P 2 | | | | 2M | | | | 1M | | | | |
| | | M.insolitus (25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | M.eboracen- sis(25) | | | | | | P 2 | | | | 1P | | | | 1M | | | | |
| | | Penaeus se- misulcatus (25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | P. monodon (25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | P.esculen- tus(25) | | | | | | 1P 2 | | | | 2M | | | | 1M | | | | |
| | | P.merguien- sis(25) | | | | | | 1M | | | | 1P | | | | 1M | | | | |
| | | P.plebejus (25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | P.latisulca- tus(25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | P.longisty- lus(25) | | | | | | 1M | | | | 2M | | | | 1M | | | | |
| | | P.latisulco- atus(26) | | | | | | | | | 1M | 1M | 1M | | | 1M | | | | |
| | | P.japonicus.125 (27) | | | | | | | | | 2P | | | | | | | | | |
| | | P.kerathurus.083 (27) | | | | | | | | | .036 2 | .206 2 | | | | | | | | |
| | Palaemonidae | | | | | | | | | | | | | | | | | | | |
| | | Palaemonetes Pugio(12) | | | | | 1M | | | 1P | 1M | 1M | 1M | | .43 7 | .30 5 | | .08 5 | | |

Subphylum

Class

Order

species^b

Group 1

| | ALDO | FUM | GDH | GOT | G-3PDH | GPDH | HK | IDH | LDH | MDH | ME | MPI | PGM | 6-PGDH | PSI | TPI | XDP |
|---|------|-----|-----|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----|----------|----------|
| Macrobrachium rosenbergii de Man (13) | | 1M | 1M | .05 2 | 1M | .02 2 | .09 2 | 1M | 1M | 2M | 1M | 1M | .26 4 | 1M | 1M | 1M | |
| Pandalidae | | | | | | | | | | | | | | | | | |
| Pandalus danae (14) | | | | | 1M | | .35 2 | | 1M | 1M | 1M | .31 2 | 1M | | | 1M | 1M |
| Pandalus jordani (14) | | | | | 1M | | .02 3 | | .15 3 | .02 3 | 1M | 1M | 1M | | | .03 3 | 1M |
| Pandalus pla- tyceros (14) | | | | | 1M | | .33 3 | | 1M | 1M | 1M | .02 2 | .11 2 | | | .02 2 | 1M |
| Pandalopsis ampla (15) | | | | | | 1P 2 | | | 1P 3 | 1M | | | 1P 3 | | | 1M | |
| Crangonidae | | | | | | | | | | | | | | | | | |
| Crangon fran- ciscorum (16) | | | | .49 2 | 1M | | 2M | .11 2 | 1M | 1M | | | .27 3 | | | .45 3 | 1M |
| Crangon nig- ricauda (16) | | | | .04 2 | 1M | | 2M | | 1M | 1M | | | .10 3 | | | .47 2 | 1M |
| Nephropidae | | | | | | | | | | | | | | | | | |
| Homarus ameri- canus (17) | 2M | | | 1M | 1M | | 3M | 1M | | .12 2 | .50 2 | | .39 2 | | | .20 3 | .03 3 |
| Homarus gamma- rus (18) | 2M | | | 1M | 1M | | 3M | 1M | | 2M | .51 4 | | .11 2 | 1M | | .08 2 | .02 2 |
| Astacidae | | | | | | | | | | | | | | | | | |
| Orconectes propinquus (19) | | | | | | | | | | .18 2 | | | .04 2 | | | .28 2 | 1M |
| Orconectes virilis (19) | | | | | | | | | | 1M | | | 2M | | | 1M | 1M |
| Orconectes immunis (19) | | | | | | | | | | 2M | | | 2M | | | 1M | 1M |
| Cambarus ro- bustus (19) | | | | | | | | | | 1M | | | 2M | | | 1M | 1M |
| Cambarus bartonii (19) | | | | | | | | | | 1M | | | 2M | | | 1M | 1M |
| Cambarus latimanus (19) | | | | | | | | | | 1M | | | 2M | | | .02 2 | 1M |
| Palinuridae | | | | | | | | | | | | | | | | | |
| Panulirus int- erruptus (16) | | | 1M | | 1M | | | | | .20 3 | | | 1M | | | .12 2 | .04 2 |
| Panulirus cygnus (16) | 1M | | | .04 2 | 1M | | .33 2 | | | .12 2 | | | .04 2 | | | .49 2 | 1M |
| Jasus edwardsii (24) | | | | | | 1M | | | | 1P 2 | 1M | | | 1M | | | |
| Jasus novae- hollandiae (24) | | | | | | 1M | | | 1P 2 | 1P 2 | 1M | | | 1M | | | |
| Scyllaridae | | | | | | | | | | | | | | | | | |
| Thenus ori- entalis (16) | 1M | | | | 1M | | 1M | | 1M | .04 2 | 1M | | 1M | 1M | | .16 3 | 1M |
| Callinassidae | | | | | | | | | | | | | | | | | |
| Callinassa californiensis (16) | | | | .46 2 | 1M | | .26 2 | 1M | 1M | .06 3 | 1M | | .13 2 | .06 3 | | .08 2 | 1M |
| Callinassa sp. (16) | | | | .07 2 | .41 2 | | | 1M | 1M | .08 2 | | 1M | .25 2 | .25 3 | | .07 2 | |
| Upogebia pugei- tensis (16) | 1M | | | 2M | 1M | 1M | .03 2 | | 1M | 1M | 1M | | .08 3 | 1M | | .07 2 | 1M |
| Galatheididae | | | | | | | | | | | | | | | | | |
| Galathea cali- forniensis (16) | | | | | 1M | | 1M | | 1M | 1M | | | 1M | | | 1M | |
| Munida hispi- da (16) | | | | | 1M | | 2M | | 1M | 2M | 1M | | .49 2 | 1M | | .05 2 | 1M |
| Munidopsis diomedea (15) | | | | | | 1P 2 | | | 1M | 1P 3 | | | 1M | | | 1P 5 | |
| Munidopsis hamata (20) | | | | | | | .36 4 | .27 2 | 1M | 1M | 1M | | .27 3 | .42 3 | | 1M | |
| Porcellanidae | | | | | | | | | | | | | | | | | |
| Pachycheles rudis (16) | 1M | | | .50 2 | | | 1M | .04 2 | 1M | 1M | 2M | | .47 2 | 1M | | .08 2 | .33 2 |

| Phylum Class Order Species ^b | Group 1 | | | | | | | | | | | | | | | | |
|--|----------|-----|-----|----------|--------|--------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----|
| | ALDO | FUM | GDH | GOT | G-3PDH | G-6PDH | HK | IDH | LDH | MDH | ME | MPI | PGM | 6-PGDH | PGI | TPI | XDH |
| Petrolisthes cinctipes(16) | 1M | | 1M | 2M | 1M | | 1M | 1M | | .04 2 | 2M | | .37 3 | | .26 3 | 1M | |
| Hippidae Emerita ana- loga(16) | 1M | | | .04 3 | 1M | | .10 2 | 1M | | .47 2 | 1M | .25 3 | .52 4 | | .20 2 | 1M | |
| Hippa pacifica (16) | | | 1M | | | | | 1M | | 2M | 1M | .08 2 | 2M | .08 2 | 1M | | |
| Paguridae Pagurus grano- simanus(16) | 1M | | | | | | .45 2 | 1M | | 1M | 1M | 1M | .10 2 | | .05 2 | 1M | 1M |
| Coenobitidae Coenobita com- presus (16) | 1M | | | | | | 1M | 1M | | 2M | 1M | .04 2 | .36 3 | .14 3 | .33 2 | | |
| Coenobita cly- peatus (16) | | | | | | | 1M | | 1M | .40 2 | | 1M | .12 3 | | .48 3 | 1M | |
| Diogeniidae Calcinus obsc- urus(16) | .04 2 | | | 1M | | | 1M | | .44 2 | .06 2 | | 1M | .12 2 | 2 M | .04 2 | 1M | |
| Calcinus tibi- oen(16) | 1M | | | 1M | 1M | | 1M | | | 1M | | 1M | .46 2 | .04 2 | .04 2 | | |
| Clibanamus panamensis(16) | | | | .13 2 | 1M | | 1M | 1M | 1M | 1M | | 1M | .61 3 | | .15 2 | 1M | |
| Clibanarius albidigilis(16) | 1M | | | | | | 1M | | 1M | .03 2 | .09 2 | 1M | .11 2 | | .06 2 | 1M | |
| Clibanarius antillensis(16) | .48 2 | | | | 1M | | 1M | 1M | 1M | .04 2 | .15 2 | .04 2 | .11 2 | 1M | .04 2 | 1M | |
| Calappidae Matu- talunaras(16) | 1M | | | 1M | 1M | | .57 3 | 1M | 1M | 2M | .26 2 | 1M | 1M | 1M | 1M | 1M | |
| Matuta planipes(16) | 1M | 1M | | .04 2 | 1M | | | 1M | 1M | 1M | 1M | 1M | .04 2 | 1M | .08 3 | 1M | |
| Cancridae Cancer gracilis (16) | | | | 1M | | 1M | 1M | 1M | | 1M | | | 1M | | .16 3 | .06 2 | 1M |
| Cancer magi- ster (16) | .05 2 | 1M | 1M | 2M | 1M | | 2M | 1M | 1M | 2M | 1M | | 1M | 1M | 1M | .24 2 | |
| Portunidae Callinectes arcuatus(16) | 1M | | | .42 3 | | | | 1M | 2M | .23 3 | | .12 2 | | | .19 2 | 1M | 1M |
| Callinectes sapidus(16) | .43 2 | | | .44 3 | 1M | | .41 2 | 1M | | .04 2 | 1M | 1M | 1M | | .08 2 | 1M | |
| Portunus sab- guinolentus(16) | 1M | | 1M | 2M | 1M | | | .31 3 | 1M | 2M | 1M | 1M | .06 3 | 1M | .04 2 | 1M | |
| Charybdis cal- lianassae(16) | 1M | | | | 1M | | 1M | | | 2M | 1M | | 1M | | .30 2 | 1M | |
| Charybdis sp.(16) | 1M | | | | 1M | | | | | 2M | 1M | | .27 2 | | 1M | 1M | |
| Xanthidae Panopeus purpureus(16) | | | | .18 2 | | | | .12 2 | | .04 2 | 1M | 1M | 1M | 1M | .08 2 | 1M | |
| Reithropanopeus harrisii(21) | | | | 1M | | | | 1M | 1M | 2M | | | 1M | 1M | 1M | | |
| Xanthodius stern- bergii(16) | | | | .05 2 | | | | 1M | | 1M | | 1M | .04 2 | 1M | 1M | 1M | |
| Ocypodidae Ocypode occi- dentalis(16) | | | | .36 3 | 1M | | | | | 1M | | 1M | 1M | 2M | 1M | 1M | |
| Ocypode quadrata(16) | | | | 2M | 1M | | | | | 2M | | 1M | .05 2 | 2M | .05 2 | 1M | |
| Uca musica(16) | | | | .05 2 | 1M | | 1M | | | .05 2 | 1M | 1M | .61 4 | 1M | .71 6 | 1M | |
| Uca princeps (16) | 1M | | | 2M | 1M | | 2M | | | 1M | .09 2 | 1M | .16 3 | 1M | .34 3 | 1M | |
| Uca spinicar- pa(22) | 1M | | 1M | | 1M | | | 1M | 1M | 3M | | 1M | .09 2 | 1M | .56 3 | | 1M |

Contd.....

| Phylum Class Order Species ^b | Group 1 | | | | | | | | | | | | | | | | |
|--|---------|-----|-----|----------|--------|-----|----|-----|-----|-----|----------|-----|----------|--------|----------|----------|----------|
| | ALDO | FUM | GDH | GOT | g-3PDH | GDH | HK | IDH | LDH | MDH | ME | MPI | PGM | 6-PGDH | PGI | TPI | XDH |
| Grapsidae | | | | | | | | | | | | | | | | | |
| Hemigrapsus oregonensis (16) | | 1M | | 1M | 1M | | 1M | 2M | | 2M | .06 2 | | .26 2 | 1M | .08 3 | 1M | .26 2 |
| Pachygrapsus crassipes (16) | | 1M | | 1M | 1M | | 1M | | | 1M | 1M | 1M | .12 2 | 1M | 1M | 1M | |
| Pachygrapsus transversus (16) | | 1M | | 1M | | | 1M | 1M | | 2M | 1M | 1M | .10 2 | | .05 2 | .02 2 | |
| Gecarcinidae | | | | | | | | | | | | | | | | | |
| Gecarcinus(6) quadratus | | | | .04 2 | | | 1M | | | 1M | .41 2 | 1M | | 1M | .04 2 | .08 2 | .04 2 |
| Sesarma | | | | | | | | | | | | | | | | | |
| Ginereum(21) | | | | 1M | | | | 1M | 1M | 2M | | | 1M | | 1M | | |

| Subphylum | Class | Order | Species ^b | ACPH | ALPH | AMY | AO' | EST | LAP | ODH | PEP | ADH | AO ⁺ | SDH | TO | PT | PYDH |
|------------------|-------|-------|----------------------|----------|----------|-----|-----------|----------|----------|----------|----------|-----|-----------------|----------|----------|----------|------|
| Group II | | | | | | | | | | | | | | | | | |
| Brachiopoda | | | | | | | | | | | | | | | | | |
| Diplostraca | | | | | | | | | | | | | | | | | |
| Conchostraca | | | | | | | | | | | | | | | | | |
| Daphnia | | | | | | | | | | | | | | | | | |
| magna(1) | | | | | | | | | | | | | | | | | |
| | | | | | .02 2 | | | .64 3 | 1M | | | | | | 1M | 1M | |
| | | | Daphnia | 2M | .61 4 | | | .34 2 | .68 3 | | | | | | 1M | 1M | |
| pulex(2) | | | | | | | | | | | | | | | | | |
| Simocephalus | | | | | | | | | | | | | | | | | |
| serrulatus(3) | | | | | | | | | | | | | | | | | |
| | | | | | .15 2 | | .57 3 | | | | | | | | | | |
| Maxillopoda | | | | | | | | | | | | | | | | | |
| Copepoda | | | | | | | | | | | | | | | | | |
| Harpacticoida | | | | | | | | | | | | | | | | | |
| Tisbe holo- | | | | | | | | | | | | | | | | | |
| thuriae(4) | | | | | | | | | | | | | | | | | |
| | | | | | .39 3 | | | 5M | .23 2 | | .59 3 | | | .32 3 | | | |
| Cirripedia | | | | | | | | | | | | | | | | | |
| Thoracica | | | | | | | | | | | | | | | | | |
| Chthamalus | | | | | | | | | | | | | | | | | |
| stellatus | | | | | | | | | | | | | | | | | |
| (Poli)(5) | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | .03 2 | | |
| Chthamalus | | | | | | | | | | | | | | | | | |
| montagu(5) | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | 1M | | |
| Chthamalus | | | | | | | | | | | | | | | | | |
| dalli Pilsbry | | | | | | | | | | | | | | | | | |
| | | | (6) | 1M | | 1M | .18 2 | .08 2 | | 1M | | | | 1M | | 1M | |
| Chthamalus | | | | | | | | | | | | | | | | | |
| fissus | | | | | | | | | | | | | | | | | |
| | | | Darwin(6) | .03 2 | | 1M | .02 3 | .03 2 | | | | | | .53 3 | 2M | | |
| Chthamalus ani- | | | | | | | | | | | | | | | | | |
| sopoma Pilsbry | | | | | | | | | | | | | | | | | |
| | | | (6) | .06 2 | | 1M | .48 2 | 1M | | | | | | 1M | 1M | | |
| Chthamalus sp. | | | | | | | | | | | | | | | | | |
| | | | (6) | 1M | | 1M | 0.02 2 | .09 2 | | | | | | .24 2 | 1M | | |
| Chthamalus sp. | | | | | | | | | | | | | | | | | |
| | | | (6) | 1M | | 1M | .02 2 | .04 3 | | | | | | .08 2 | .02 2 | | |
| Balanus amphi- | | | | | | | | | | | | | | | | | |
| trite amphi- | | | | | | | | | | | | | | | | | |
| trite Darwin(7) | | | | | | | | | | | | | | | | | |
| | | | 1M | | | | 4M | 1M | | 1M | | | | | 1M | | |
| Balanus amphi- | | | | | | | | | | | | | | | | | |
| trite inexpect- | | | | | | | | | | | | | | | | | |
| atus Pilsbry(7) | | | | | | | | | | | | | | | | | |
| | | | | | | | .38 2 | .46 2 | | 1M | | | | .04 2 | .06 3 | | |
| Balanus glandula | | | | | | | | | | | | | | | | | |
| | | | Darwin(7) | 2M | .15 2 | | .74 5 | .22 3 | 1M | .40 3 | | | | .24 3 | .04 2 | | |
| Balanus crenatus | | | | | | | | | | | | | | | | | |
| | | | Brugiere(7) | 1M | | | .33 2 | .38 5 | | .12 2 | | | | .20 4 | | | |
| Semibalanus | | | | | | | | | | | | | | | | | |
| cariosus(Pallas) | | | | | | | | | | | | | | | | | |
| | | | (7) | | | | 2M | | | | | | | .50 2 | .04 3 | | |
| Hoplocarida | | | | | | | | | | | | | | | | | |
| Hoplocarida | | | | | | | | | | | | | | | | | |
| Stomatopoda | | | | | | | | | | | | | | | | | |
| | | | Squilla nepa(8) | .08 2 | | | .03 2 | .05 2 | | 1M | | | | 1M | | .10 2 | |
| Squilla wood- | | | | | | | | | | | | | | | | | |
| masoni(8) | | | | | | | | | | | | | | | | | |
| | | | | 3M | | | 1M | | | 1M | | | | 1M | | | |
| Eumalacostraca | | | | | | | | | | | | | | | | | |
| Peracarida | | | | | | | | | | | | | | | | | |
| Isopoda | | | | | | | | | | | | | | | | | |
| Excitrolana | | | | | | | | | | | | | | | | | |
| | | | kincaidii(7) | | .22 2 | | | .04 2 | .10 2 | 1M | | | | | | | |
| Excitrolana | | | | | | | | | | | | | | | | | |
| | | | sp.(7) | | 1M | | | 2M | | 1M | | | | | | | |
| Eucarida | | | | | | | | | | | | | | | | | |
| Euphausiacea | | | | | | | | | | | | | | | | | |
| Euphausia super- | | | | | | | | | | | | | | | | | |
| | | | ba(9) | 3M | 1M | | .08 3 | .26 3 | 1M | .08 3 | | | | | .02 2 | | |
| Euphausia | | | | | | | | | | | | | | | | | |
| | | | mucronata(10) | .13 3 | .33 2 | | .13 2 | .22 3 | 1M | .06 3 | | | | | 2M | | |
| Euphausia dist- | | | | | | | | | | | | | | | | | |
| | | | inguenda (10) | .27 5 | .60 3 | | .23 4 | .23 3 | 1M | .13 4 | | | | | 2M | | |

| Class Order Species ^b | ACPH | APLH | AMY | AO ¹ | EST | LAP | ODH | PEP | ADH | AO ² | SDH | TO | PT | FYDH |
|--|----------|------|-----|-----------------|------------|----------|----------|----------|-----|-----------------|--------|--------|----|--------|
| Decapoda | | | | | | | | | | | | | | |
| Penaeidae | | | | | | | | | | | | | | |
| Penaeus mer- guensis de Man (8) | 1M | 1M | | 1M | | 1M | | | | | | | 4M | |
| Penaeus aztecus Ives (11) | 1M | | | 2M | .35 3 | .06 3 | | .08 3 | | | | | 2M | |
| Penaeus duo- rarum Burken- road (11) | 1M | | | 2M | .52 5 | 1M | | .09 3 | | | | | 2M | |
| Penaeus seti- ferus Linnaeus (11) | 1M | | | 2M | .22 4 | 1M | | .07 3 | | | | | 2M | |
| Palaemonidae | | | | | | | | | | | | | | |
| Palaemonetes pugio(12) | | 1P? | | | .39 2 | 1M | | | | | | | 2M | |
| Penaeus sty- lirostris(23) | 2M | | | | 1P | | | | | 2M | | | | |
| Penaeus vannamei(23) | 1M | | | | 2 P 2 | | | | | 1M | | | | |
| Metapenaeus macleayi(25) | | 2M | | | 1M | | P | | | | P 2 | M | | |
| M.bennettiae (25) | | 2M | | | 1M | | P 2 | | | | 1M | P 2 | | |
| M. endeavouri (25) | | 1M | | | 1M | | P 3 | | | | M | P 2 | | |
| M. ensis(25) | | 1M | | | 1M | | 1M | | | | P 2 | M | | |
| M. insolitus (25) | | 2M | | | 1M | | P 2 | | | | P 2 | M | | |
| M. eboracensis (25) | | 2M | | | 1M | | P 2 | | | | M | M | | |
| Penaeus semi- sulcatus(25) | | 2M | | | 1M | | P 2 | | | | M | M | | 1M |
| P. monodon(25) | | 1M | | | 1M | | 1M | | | | M | M | | 1M |
| P. esculen- tus(25) | | 1M | | | 1M | | P 3 | | | | P 2 | M | | 1M |
| P. merguien- sis(25) | | 2M | | | 1M | | M | | | | M | M | | 1M |
| P. plebejus (25) | | 2M | | | 1M | | P 2 | | | | M | M | | P 2 |
| P. latisul- catus(25) | | 1M | | | 1M | | P 2 | | | | M | M | | M |
| P. longisty- lus (25) | | 2M | | | 1M | | P 2 | | | | M | M | | M |
| P. latisul- catus(26) | | | | | | | 1M | | 1M | | | | | |
| P. japonicus (27) | | | | | 0.429 2 | | | | | .077 2 | | | | |
| P. kerathurus (27) | | | | | .125 .2 | | | | | | | | | |
| Macrobrachium rosenbergii de Man(12) | .07 3 | | | 1M | | .38 2 | | | | | | 1M | 7M | |
| Pandalidae | | | | | | | | | | | | | | |
| Pandalus danae(14) | 3M | | | 3M | 4M | .14 2 | | 1M | | | | 1M | 5M | |
| Pandalus jordani(14) | .02 3 | | | 2M | .02 2 | .12 4 | | 2M | | | | 1M | 4M | |
| Pandalus platyceros(14) | .25 3 | | | .07 2 | .07 2 | .11 2 | | 1M | | | | 1M | 4M | |
| Pandalopsis ampla(15) | | | | | 3M | 1M | | 1P 2 | | | | 1M | 4M | |
| Crangonidae | | | | | | | | | | | | | | |
| Crangon fran- ciscorum(16) | 2M | | | .10 2 | .10 2 | 1M | .10 2 | | | | .11 | 3M | 4M | |

| Subphylum | Class | Order | Species ^b | ACPH | ALPH | AMY | AO ^c | EST | LAP | ODH | PEP | ADH | AO ^d | SDH | TO | PT | PY |
|-----------|-------|--------------------------------|-------------------------|----------|------|----------|-----------------|----------|----------|----------|-----|-----|-----------------|----------|----------|----------|----|
| | | | Crangon nigricauda (16) | .47 2 | | | .13 2 | .35 2 | 1M | .03 2 | | | | 1M | 3M | 4M | |
| | | Nephropidae | | | | | | | | | | | | | | | |
| | | Homarus americanus (17) | .31 2 | | | | .33 2 | .37 3 | 1M | | | | | | 5M | 6M | |
| | | Homarus gamma-rus (18) | .42 2 | | | | 1M | .47 2 | | | | | | | 5M | 6M | |
| | | Astacidae | | | | | | | | | | | | | | | |
| | | Orconectes propinquus (19) | 1M | | 2M | | .15 2 | 3M | .46 3 | 1M | | | | | 2M | 5M | |
| | | Orconectes virilis (19) | 1M | | | .02 2 | 4M | 1M | .44 2 | 1M | | | | | 1M | 2M | |
| | | Orconectes immunis (19) | 1M | | 2M | | .23 4 | .42 3 | | 1M | | | | | 1M | 2M | |
| | | Cambarus robustus (19) | 1M | | 1M | | .50 4 | 2M | .18 2 | 1M | | | | | 1M | 4M | |
| | | Cambarus bartonii (19) | 1M | | 1M | | .50 2 | 1M | .49 2 | 1M | | | | | .16 2 | 4M | |
| | | Cambarus latimanus (19) | 1M | | | | .46 3 | | .44 2 | 1M | | | | | 1M | 5M | |
| | | Palinuridae | | | | | | | | | | | | | | | |
| | | Panulirus interruptus (16) | 3M | | | | | .19 2 | 1M | .06 2 | | | | | .44 3 | 6M | |
| | | Panulirus cygnus (16) | 4M | | | | .49 2 | .19 2 | 1M | | | | | | 1M | 6M | |
| | | Jasus edwardsii (24) | P 2 | | | | | P 2 | | | | | | 1M | 2M | | |
| | | Jasus novae hollandiae (24) | | P 2 | | | | P 2 | | | | | | 1M | 2M | | |
| | | Scyllaridae | | | | | | | | | | | | | | | |
| | | Thenus orientalis (16) | 2M | | | | .04 2 | .48 2 | 1M | | | | | 1M | | .09 2 | |
| | | Callinassidae | | | | | | | | | | | | | | | |
| | | Callinassa californiensis (16) | 2M | | | | .16 2 | .66 4 | 1M | 1M | | | | .16 2 | 3M | 5M | |
| | | Callinassa sp. (16) | | 3M | | | .13 2 | .46 4 | .67 4 | | | | | | 2M | | |
| | | Upogebia pugetensis (16) | 3M | | | | .49 2 | .33 3 | 1M | .6 2 | | | | .22 2 | .43 3 | 4M | |
| | | Galatheidae | | | | | | | | | | | | | | | |
| | | Galathea californiensis (16) | .58 4 | | | | .30 2 | .12 2 | .42 4 | | | | | 1M | | 3M | |
| | | Munida hispida (16) | .27 3 | | | | .05 2 | .25 2 | .50 2 | .13 2 | | | | | 1M | 4M | |
| | | Munidopsis diomedea (15) | | | | | | 2M | | | 1M | | | | | 3M | |
| | | Munidopsis hamata (20) | 2M | .47 2 | | | 1M | .07 2 | .52 3 | | 3M | | | | 3M | | |
| | | Porcellanidae | | | | | | | | | | | | | | | |
| | | Pachycheles rudis (16) | 2M | | 1M | | | .04 2 | 2M | | | | | | 1M | 4M | |
| | | Petrolisthes cinctipes (16) | .49 2 | | | | 1M | .32 2 | | 1M | | | | | 3M | 3M | |
| | | Hippidae | | | | | | | | | | | | | | | |
| | | Emerita analoga (16) | 1M | | | | .09 2 | .28 2 | | | | | | | 2M | 2M | |
| | | Hippa pacifica (16) | 1M | 2M | | | | 4M | 1M | | | | | | 1M | | |
| | | Paguridae | | | | | | | | | | | | | | | |
| | | Pagurus granosimanus (16) | .05 2 | | | | 1M | .44 2 | 1M | .05 2 | | | | | 1M | 2M | |
| | | Coenobitidae | | | | | | | | | | | | | | | |
| | | Coenobita compressus (16) | | | | | 2M | 3M | .55 5 | | | | | | | 3M | |
| | | Coenobita clypeatus (16) | | | | | 2M | 1M | | | | | | | | 1M | |
| | | Diogenidae | | | | | | | | | | | | | | | |

| Subphylum Class Order Species ^b | Group II | | | | | | | | | | | | | | |
|---|----------|----------|----------|-----------------|----------|----------|----------|-----|-----|-----------------|-----|-----|----------|------|--|
| | ACH | ALPH | AMY | AO ¹ | EST | LAP | ODH | FEP | ADH | AO ² | SDH | TO | PT | PYDH | |
| Calcinus tibicen(16) | .04 2 | 1M | 1M | 1M | .25 2 | 1M | | | | | | | 1M | | |
| Clibanarus panamensis(16) | 3M | .47 2 | 1M | .54 3 | | | 1M | | | | | 1M | 1M | | |
| Clibanarius albidguis(16) | .05 2 | 3M | 1M | .03 2 | 2M | | 1M | | | | | | 1M | | |
| Clibanarius antillensis(16) | 2M | | 1M | 1M | 6M | .06 2 | | | | | | | 1M | | |
| Calappidae | | | | | | | | | | | | | | | |
| Matuta lunaris (16) | 1M | | | 1M | 4M | | 1M | | | | | | 6M | | |
| Matuta planipes (16) | .05 2 | | | 2M | 3M | | 1M | | | | | | 3M | | |
| Canceridae | | | | | | | | | | | | | | | |
| Cancer gracilis (16) | | | | .28 2 | .52 3 | 1M | 1M | | | | 1M | 2M | 3M | | |
| Cancer magister (16) | | | | 1M | .11 2 | 1M | 1M | | | | | 3M | 5M | | |
| Portunidae | | | | | | | | | | | | | | | |
| Callinectes arcuatus(16) | 1M | | | .45 2 | .48 3 | | .23 2 | | | | | 1M | 2M | | |
| Callinectes sapidus(16) | 1M | | 1M | 2M | .46 3 | 1M | 1M | | | | | 1M | 2M | | |
| Portunus sanguinolentus(16) | | | | .48 3 | .23 2 | .66 3 | 1M | | | | | 3M | .17 2 | | |
| Charybdis callianassae(16) | 1M | | | 3M | .4M | .06 2 | | | | | | | .24 2 | | |
| Charybdis sp. (16) | | | | 1M | .11 2 | | | | | | | | .33 2 | | |
| Xanthidae | | | | | | | | | | | | | | | |
| Panopeus purpureus(16) | .54 4 | .26 3 | | .29 2 | 6M | | | | | | | 1M | 3M | | |
| Rhithropanopeus harrisi(16) | | 1M | | | 3M | | | 2M | | | | | | | |
| Xanthodius sternbergii(16) | | 1M | .32 2 | .27 2 | | | 1M | | | | | 2 M | 2M | | |
| Ocypodidae | | | | | | | | | | | | | | | |
| Ocypode occidentalis(16) | | 2M | | .18 2 | .18 2 | .19 3 | | | | | | | 2M | | |
| Ocypode quadrata (16) | | 2M | | .13 2 | .05 2 | 3M | | | | | | | 2M | | |
| Uca musica(16) | 1M | .77 6 | | .04 2 | 2M | 1M | | | | | | | 2M | | |
| Uca princeps(16) | 1M | 1M | | 1M | .08 2 | 1M | 1M | | | | | | 3M | | |
| Uca speciosa(16) | 1M | | | | 4M | | | | | | | 1M | .42 2 | | |
| Uca spinicarpa (22) | 1M | | | | 4M | | | | | | | 1M | .09 2 | | |
| Grapsidae | | | | | | | | | | | | | | | |
| Hemigrapsus oregonensis(16) | .06 2 | | 2M | 2M | .04 2 | 1M | 1M | | | | 1M | | .43 2 | | |
| Pachygrapsus crassipes(16) | .23 2 | .04 2 | 2M | 2M | 2M | | | | | | | | 3M | | |
| Pachygrapsus transversus(16) | .42 2 | .02 2 | 2M | 4M | .34 4 | | | | | | | | 2M | | |
| Gecarcinidae | | | | | | | | | | | | | | | |
| Gecarcinus auadratus(16) | | .05 2 | | 3M | .09 2 | 1M | | | | | | | 4M | | |
| Sesarma cinereum(21) | | 1M | | | 3M | | | 1M | | | | 1M | | | |

^aProportion of individuals heterozygous and number of alleles given for loci classified as polymorphic, P; monomorphic loci designated as "M" with prefix indicating number of loci. For each polymorphic protein the most heterozygous locus is listed in the body of the table. Enzymes grouped according to Gillespie and Kojima (1968; see text for additional references). Summary measures of variation defined in text: n_a , average number of allozymes detected per locus; P, proportion of loci polymorphic; H, average proportion of heterozygous individuals per locus. Abbreviations for proteins are as follows: Group I-ALDO, Aldolase; FUM Fumarase; G6-PDH, Glycose-6-Phosphate dehydrogenase; GDH, glutamate dehydrogenase; GOT glutamate-oxaloacetate transaminase; G-3PDH, glyceraldehyde-3-phosphate dehydrogenase; GPDH, glycerophosphate dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; HK, hexokinase; IDH-isocitrate, dehydrogenase; LDH, lactate dehydrogenase; MDH, Malate dehydrogenase; ME, malic enzyme (NADP-dependent MDH); MPI, mannose-phosphate isomerase; PGM, Phosphoglucmutase; 6-PGDH, 6-phosphogluconate dehydrogenase; PGI phosphoglucose (phosphohexose) isomerase; TPI, triosephosphate isomerase; XDH, xanthine dehydrogenase. Group II - ACPH, acid phosphatase; ADH, alcohol dehydrogenase; ALPH, Alkaline phosphatase; AMY, amylase; AO, acetaldehyde oxidase; EST, esterase; LAP, leucine aminopeptidase; ODH Octanol dehydrogenase, PEP peptidase; ADH, alcohol dehydrogenase; ALPH, alkaline phosphatase; AMY, amylase; AO, acetaldehyde oxidase; EST, esterase; LAP, leucine aminopeptidase; GDH, octanol dehydrogenase, PEP peptidase; PER peroxidase, SDH, sorbitol (inositol) dehydrogenase. Other-TO, letrozolium oxidase (superoxidase dismutase); PT, proteins revealed by nonspecific general protein stain.

^bReferences: (1) Hebert, 1974c; (2) Berger and Sutherland, 1978; (3) Smith and Fraser, 1976; (4) Battaglia et al., 1978a; (5) Dando et al., 1979; (6) Hedgecock, 1979; (7) D. Hedgecock and K. Nelson, unpublished data cited in Hedgecock et al., 1982 (8) Redfield et al., 1980; (9) Ayala et al., 1975; (10) Ayala and Valentine, 1979; (11) Lester, 1979; (12) Fuller and Lester, 1980; (13) Hedgecock et al., 1979; (14) Berthelemy, 1978; (15) Gooch and Schopf, 1973; (16) Nelson and Hedgecock, 1980; (17) Tracey et al., 1957; (18) Hedgecock et al., 1977; (19) Nemeth and Tracey, 1979; (20) Costa and Bisol, 1978; (21) Gooch, 1977; (22) Salmon et al., 1979; (23) Lester, 1983; (24) Smith et al., 1980; (25) Mulley and Latter, 1980; (26) Richardson, 1982; (27) Dematthaeis et al., 1983;

^cThese averages include summary measures of variation reported for four harpacticoid copepods and a gammarid amphipod (Battaglia et al., 1978a), another gammarid amphipod (Battaglia et al., 1978a), another gammarid amphipod (Gooch and Hetrick, 1979) and three thoracican barnacles (Nevo, 1978).

The table would show that only little work has been carried out on shrimps to find out the genetic structure of their populations.

Out of 31 enzymes studied so far in crustaceans as given in the above table, 15 enzymes have been related for the present study on shrimps and the results on these isozymes studies are given in the following sections.

Results:**Genetic basis of the observed electrophoretic variation:**

DNA (Deoxyribonucleic acid) is a giant molecule composed of a sequence of subunits called nucleotides attached to a sugar-phosphate backbone. Triplet combination of these nucleotides are the genetic code for each amino acid, which forms the backbone of protein molecule. A segment of DNA which codes for a single amino acid sequence is called a gene. The location of that segment of DNA in relation to total DNA is called the locus for that gene. These segments of DNA which codes for specific polypeptides are grouped together to form chromosomes. In diploid organism two copies of chromosomes are present and result in two genes per locus per individual. Different genes that occur in the same locus are called alleles. Alleles differ from one to other by minor amino acid substitutions in the polypeptides. When alleles in a locus are products of similar base sequence it will have similar allele combination termed homozygote. However if the base sequence differed slightly in a locus, the product will be a combination of two different alleles called heterozygote. A locus is considered to be polymorphic when frequencies of the common allele was not greater than 0.95 and thus reveal electrophoretic variants. Enzyme Loci in which all specimens

tested appeared as a single monomorphic band were classified as monomorphic loci. Those with two or more well separated zones of activity are assumed to be the products of two or more loci. The enzyme loci in different tissues tested were numbered consecutively from the most cathodal to the most anodal and alleles were designated alphabetically.

Electrophoresis conducted in the present investigation helped to detect allelic and non-allelic forms of proteins in each individual of the species tested and thus enabled to find out the differences between heterozygotes and homozygotes and to quantify the number of individuals with each respective genotypes for the analysis of the genetic structure of each population sample drawn from Penaeus indicus and Parapenaeopsis stylifera species. (Table 44-47)

Genetic variation studies were carried out in fifteen enzymes, such as acid phosphatase, alcohol dehydrogenase, aldehydeoxidase, aldolase, alkaline phosphatase, esterase, ~~alpha~~-glycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, malic enzyme octanol dehydrogenase, 6-phosphogluconate dehydrogenase, 1-pyrroline dehydrogenase, sorbitol dehydrogenase, and tetrazolium oxidase in different tissues like eye, hepatopancreas and muscle of Penaeus indicus and Parapenaeopsis stylifera (Table 69, 70). Alternative

enzyme forms derived from different loci are isozymes where as multiple enzymes encoded by alternative alleles at a locus are allozymes.

In the case of monomeric proteins each homozygote showed one protein band in the gel after staining. When single banded allozymes were electrophoretically different, the homozygotes differed in their location in the gel. In heterozygote both products of the homozygotes were formed and thus 2 bands were observed in the gel.

In the dimeric proteins two polypeptide chains are present in the protein globule and hence three bands are generally found in the heterozygous individuals.

Acid phosphatase:

Penaeus indicus

Expression of this enzyme in eyes, hepatopancreas and muscle was analysed. According to their relative mobility, and staining intensity of the bands five zones of enzyme activity were observed. Numbering of bands was done from the cathodal end i.e. from slowest migrating band, to anodal end i.e. fastest migrating band. Thus four loci (Acph 1, 2, 4, 5) were identified in hepatopancreas whereas muscle showed Acph-3 locus and eye Acph-1 and Acph-2 locus. Expression

observed in different tissues tested is given in Fig. 13. Genetic variation study was carried out in muscle and eye tissues of the P. indicus population samples collected from four different areas viz. Cochin, Tuticorin, Madras and Waltair. But Acph-3 locus was found to be polymorphic only in muscle tissue analysed in all the localities. A two banded phenotype of presumed heterozygotes is consistent with a monomer subunit structure of the enzyme found with 2 alleles (Table 48). These two alleles, one slow moving and the other fast moving were designated as A and B respectively (Fig. 14; Plate 15). A and B frequencies of these two alleles are given in the table 71. Gene frequency of dominant allele is some what similar.

Lack of Hardy-Weinberg equilibrium was noticed due to heterozygote deficiency and excess of homozygotes at all stations. Observed and expected frequency of heterozygote were calculated (Table 73).

P. stylifera:

Acid phosphatase was analysed for genetic variation in P. stylifera collected from Cochin and Bombay. Four different zones of activity two regions in hepatopancreas and one region in muscle and eye tissues was observed (Fig.15). Thus the two regions seen in hepatopancreas represent Acph-3

Table 44: Relative mobility (R.M.) values with their intensities of different enzymatic proteins analysed in different tissues of Penaeus indicus.

| Enzyme | Tissue | RM | Intensity |
|------------------------|----------------|-----------|-----------|
| Malic enzyme | Eye | 8.3-10 | xx |
| | Muscle | 21.7-26.6 | x |
| Malate dehydrogenase | Eye | 23.3-28.3 | xx |
| | | 26.7-33.3 | x |
| | | 40-50 | x |
| Lactate dehydrogenase | Eye | 25-28.3 | x |
| | Hepatopancreas | 8.3-11.7 | x |
| | Muscle | 8.3-11.7 | x |
| | | 25-28.3 | xx |
| Sorbitol dehydrogenase | Hepatopancreas | 18.3-22 | xx |
| | Muscle | 13.3-16.7 | xx |
| Aldehydeoxidase | Hepatopancreas | 17.2-20 | xx |
| | | 28.3-30 | xx |
| | Muscle | 33-36.7 | xx |
| Alcoholdehydrogenase | Eye | 13.3-18.3 | xx |
| | | 28.3-35 | xx |
| | Hepatopancreas | 8-12 | x |
| | | 13.3-16.7 | xx |
| | Muscle | 13.3-16.7 | xx |
| Acid Phosphatase | Eye | 6.7-10 | xx |
| | | 26.7-33.3 | xx |
| | Hepatopancreas | 6.7-10 | xx |
| | | 26.7-33.3 | xx |
| | | 66.7-75 | x |
| | | 80-86.7 | xx |
| | Muscle | 33-36 | xx |
| Tetrazolium oxidase | Eye | 5-7 | |
| | | 23.3-28.3 | |
| | Hepatopancreas | 33.3-38 | |
| | | 41.7-46.7 | |
| | Muscle | 23.3-26.7 | |

Contd....

| Enzyme | Tissue | RM | Intensity |
|--------------------------------------|--------------------------|----------|-----------|
| 1-Pyrroline dehydrogenase | Hepatopancreas | 36.7-40 | xx |
| | Muscle | 16.7-20 | xx |
| 6-Phosphogluconate dehydrogenase | Eye | 8.3-11.7 | x |
| | | 30-40 | x |
| | Hepatopancreas Muscle | 31-33.3 | xx |
| | | 33-41.7 | x |
| Octanol dehydrogenase | Eye | 17-20 | xx |
| | Hepatopancreas | 15-18.3 | xx |
| | Muscle | 23.3-30 | xx |
| Alpha Glycerophosphate dehydrogenase | Hepatopancreas | 20-23 | xx |
| Aldolase | Muscle | 18.3-20 | xx |

Table 45: Relative mobility (RM) with their intensities for the different genotypes of various enzymes analysed in Penaeus indicus.

| Enzyme | Locus | Genotype | RM | Intensity |
|-----------------------|--------|----------|-----------|-----------|
| Acid Phosphatase | Acph-3 | AA | 25-27.3 | XX |
| | | AB | 25-27.3 | X |
| | | | 34-36.3 | X |
| | | BB | 34-36.3 | XX |
| Aldehyde oxidase | Ao-1 | AB | 16.7-20 | X |
| | | | 21.7-25 | X |
| | | | 28.3-30 | XX |
| | | AA | 16.7-20 | XX |
| | | | 28.3-30 | XX |
| | | AB | 16.7-20 | X |
| | | | 21.7-25 | X |
| | | | 28.3-30 | XX |
| | | BB | 21.7-25 | XX |
| | | | 28.3-30 | XX |
| Aldolase | Ald-1 | AA | 18.3-20 | XX |
| | | AB | 18.3-20 | X |
| | | | 21.0-23.3 | X |
| | | BB | 21.0-23.3 | XX |
| | | BC | 21.0-23.3 | X |
| | | | 25-26.7 | X |
| | | CC | 25-26.7 | X |
| Alkaline phosphatase | Alph- | AA | 50-53.3 | XXX |
| | | AB | 50-53.3 | XX |
| | | | 55-58.3 | XX |
| | | BB | 55-58.3 | XXX |
| Octanol dehydrogenase | Odh-2 | AA | 17.7-20 | XX |
| | | AB | 17.7-20 | X |
| | | | 23.3-25.3 | XX |
| | | | 28-30.3 | X |
| | | BB | 28-30.3 | XX |

Contd.....

| Enzyme | Locus | Genotype | RM | Intensity |
|----------------------------------|----------|----------|-----------|-----------|
| 6-Phosphogluconate dehydrogenase | 6-Pgdh-2 | AA | 31.0-33.3 | xx |
| | | AB | 31.0-33.3 | x |
| | | | 35-36.7 | x |
| | | BB | 35-36.7 | xx |
| Malate dehydrogenase | Mdh-2 | AA | 31.7-36.7 | xx |
| | | | 23-28 | x |
| | | | 31.7-36.7 | x |
| | | BB | 23-28 | xx |
| Malic enzyme | Me-1 | AA | 8.3-10 | xx |
| | | AB | 8.3-10 | x |
| | | | 15-16.7 | x |
| | | BB | 15-16.7 | xx |

Table 46: Relative mobility (RM) with their intensities of different enzymatic proteins analysed in different tissues of Parapenaeopsis stylifera.

| Enzyme | Tissue | RM | Intensity |
|--------------------------------|----------------|-----------|-----------|
| Alcohol dehydrogenase | Eye | 15-18.3 | xx |
| | Hepatopancreas | 30-33.3 | xx |
| | Muscle | 15-18.3 | xx |
| Octanol dehydrogenase | Eye | 30-33.3 | xx |
| | Hepatopancreas | 30-33.3 | x |
| | | 40-45 | xx |
| | Muscle | 13.3-16.7 | xx |
| | | 28.3-31.7 | x |
| Glycerophosphate dehydrogenase | Hepatopancreas | 16.7-20 | xx |
| Malate dehydrogenase | Eye | 20-25.3 | xx |
| | Muscle | 41.7-48.3 | x |
| Acid Phosphatase | Eye | 8.2-13.3 | xx |
| | Hepatopancreas | 30-33.3 | xx |
| | | 66.7-70 | x |
| | Muscle | 15.2-17 | xx |
| Alkaline phosphatase | Eye | 21.7-25 | x |
| | Hepatopancreas | 46.7-50 | xx |
| | Muscle | 30-33 | xx |
| Tetrazolium oxidase | Eye | 3.3-8.3 | |
| | Hepatopancreas | 3-5 | |
| | | 25-30.3 | |
| | Muscle | 3.3-8.3 | |
| Aldehydeoxidase | Hepatopancreas | 18.3-21.7 | xx |
| | | 31.7-35 | x |
| | | 6.7-8.3 | x |
| Malic enzyme | Eye | 8.3-10 | x |
| | Muscle | 10-13.3 | x |

Contd...

| Enzyme | Tissue | RM | Intensity |
|---------------------------|--------------|---------|-----------|
| Esterase | Eye | 10-13.3 | xx |
| | | 18.3-20 | xx |
| | | 30-35.3 | x |
| | Hepatopaneas | 8-12.3 | xx |
| | | 30-33.3 | x |
| | Muscle | 18.3-20 | xx |
| 1-Pyrroline dehydrogenase | Hepatopaneas | 16.7-20 | x |
| | Muscle | 16.7-20 | x |

Table 47: Relative mobility (RM) with their intensities for the different genotypes of various enzymes analysed in Parapenaeopsis stylifera

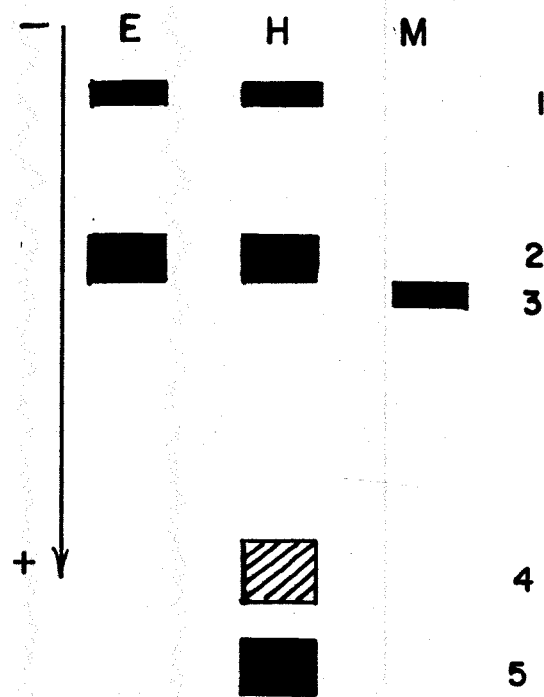
| Enzyme | Locus | Geno- type | RM | Intensity |
|--------------------------|--------|---------------|---------|-----------|
| Acid Phosphatase | Acph-2 | AA | 15.3-17 | XX |
| | | AB | 15.3-17 | X |
| | | | 18.4-20 | X |
| | | BB | 18.4-20 | XX |
| Alkaline phosphatase | Alph-2 | AA | 25-27.2 | XX |
| | | AB | 25-27.2 | X |
| | | | 29-30.5 | X |
| | | BB | 29-30.5 | XX |
| Alcohol dehydrogenase | Adh-2 | AA | 30-32.2 | XX |
| | | AB | 30-32.2 | X |
| | | | 34-36.2 | X |
| | | BB | 34-36.2 | XX |
| Esterase | Est-1 | | 10-13.3 | XX |
| | Est-2 | AA | 22.2-24 | XX |
| | | AB | 18.3-20 | X |
| | | | 22.2-24 | X |
| | | BB | 18.3-20 | XX |
| | Est-3 | | 30-35.3 | X |
| | | | | |
| Malatedehydro- genase | Mdh-2 | AA | 20-25.2 | XX |
| | | AB | 20-25.2 | X |
| | | | 38.3-43 | X |
| | | BB | 38.3-43 | XX |
| Tetrazolium oxidase | To-1 | AA | 3.3-5 | |
| | | AB | 3.3-5 | |
| | | | 6.2-8 | |
| | | BB | 6.2-8 | |
| | To-2 | CC | 25.2-30 | |
| | | CD | 25.2-30 | |
| | | | 33.3-38 | |

Table 48: Observed and expected phenotype frequency of acid phosphatase (Acph-3) observed in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | χ^2 |
|-----------|----------|------|------|-----|----------|
| Cochin | Observed | 18 | 2 | 16 | 28.6 |
| | Expected | 10 | 18 | 8 | |
| Tuticorin | Observed | 17 | 2 | 17 | 28.4 |
| | Expected | 9 | 18 | 9 | |
| Madras | Observed | 21 | 1 | 14 | 30.6 |
| | Expected | 13 | 17 | 6 | |
| Waltair | Observed | 23 | 1 | 12 | 32.1 |
| | Expected | 25.3 | 16.3 | 4.3 | |

Fig. 13. Expression of acidphosphatase in different tissues of Penaeus indicus.

Fig. 13



E - Eye

H - Hepatopancreas

M - Muscle

**Fig. 14. Expression of different genotypes of
acid phosphatase (Acph-3) in muscle
tissue of Penaeus indicus**

**Fig. 15. Expression of acid phosphatase in
different tissues of Parapenaeopsis
stylifera.**

Fig. 14

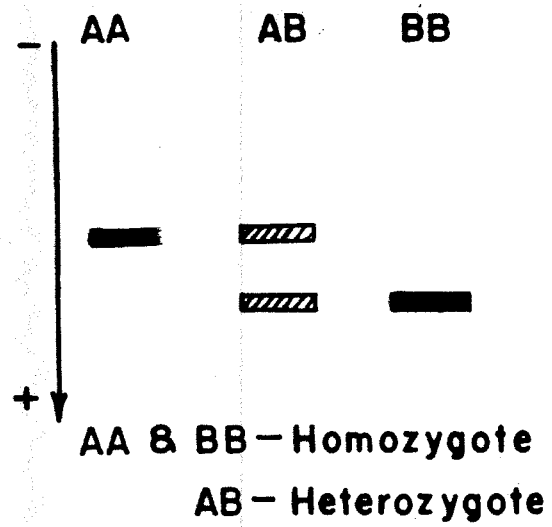


Fig. 15

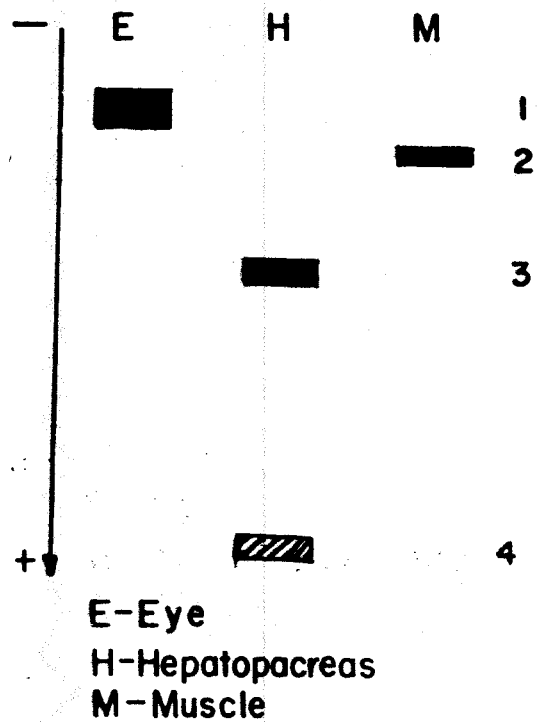


Fig. 16. Expression of different genotypes of acid phosphatase (Acph-2) in muscle tissue of Parapenaeopsis stylifera.

Fig. 17. Expression of alcohol dehydrogenase in different tissues of Penaeus indicus.

Fig.16

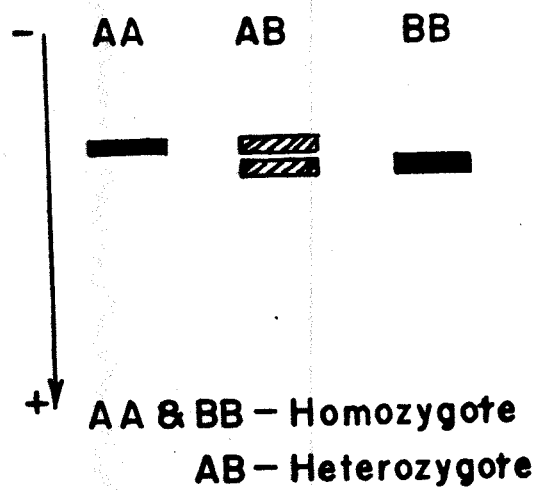


Fig.17

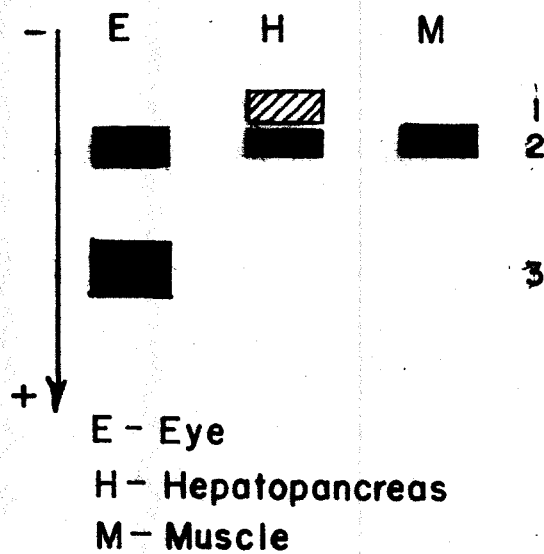


Plate 15: Showing the different genotypes of acid phosphatase (Acph-3) in muscle tissues of Penaeus indicus AA, BB,-homozygotes AB-heterozygote.

Plate 16: Showing the different genotypes of acid phosphatase (Acph-2) in muscle tissues of Parapenaeopsis stylifera, AA, BB-homozygotes, AB-Heterozygote.

and Acph-4 whereas in muscle and eye showed Acph-2 and Acph-1 respectively. Genetic analysis was carried in eye and muscle tissues. Acph-2 locus in muscle tissue exhibited two banded heterozygote expression indicating monomer structure of the enzymes. Genetic variation studies in these tissues revealed Acph-2 found in muscle to express polymorphism in all the localities (Fig. 16; Plate 16).

The allele frequency observed is given in Table 72. There seems to be significant difference in the phenotypic distribution and allele frequency between the samples of Cochin and Bombay. Expected and observed phenotypic expression were seen in Table 49. Observed and expected of heterozygote was also found out (Table 74).

Alcohol dehydrogenase:-

Phenæus indicus

Samples for genetic variation studies of P. indicus were collected from Cochin, Tuticorin, Madras and Waltair. Alcohol dehydrogenase activity appeared in three zones which were coded by three genetic loci (Fig. 17). Eye tissue showed 2 loci one fast migrating Adh-3 locus and one slow migrating Adh-2 locus whereas hepatopancreas expressed the Adh-1 and Adh-2 locus and muscle with Adh-2 locus. The Adh-2 locus represented in all tissues. Genetic variation analysis was

Table 49: Observed and Expected phenotype frequency of acidphosphatase (Acph-2) observed in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|------|------|------|---------------|
| Cochin | Observed | 25 | 3 | 8 | 22.3 |
| | Expected | 19.5 | 14 | 2.5 | |
| Bombay | Observed | 11 | 2 | 22 | 24.2 |
| | Expected | 4.3 | 16.3 | 15.3 | |

Table 50: Observed and expected phenotype frequency of alcohol dehydrogenase (Adh-2) observed in Parapenaeopsis stylifera with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|------|-----|-----|---------------|
| Cochin | Observed | 34 | 0 | 2 | 40.025 |
| | Expected | 32 | 3.8 | .1 | |
| Bombay | Observed | 32 | 1 | 3 | 29.04 |
| | Expected | 29.3 | 6.3 | 0.3 | |

carried out in muscle and hepatopancreas tissues. Allele frequency calculated showed the common allele to have value more than 0.95. Thus these loci were considered to be monomorphic.

Parapenaeopsis stylifera:

In Parapenaeopsis stylifera also alcohol dehydrogenase was employed for the genetic variation analysis for the samples collected from two localities viz. Cochin and Bombay. Two zones of enzymes activity expressed the presence of loci Adh-2 in hepatopancreas and Adh-1 in eye and muscle tissues (Fig.18) Genetic variation studies in eyes and hepatopancreas confirmed Adh-2 locus in hepatopancreas to be polymorphic. Different genotype pattern observed is given in Fig. 19(Plate 17).

Phenotypic expression observed is given in Table 50. The allele frequency was calculated and didn't express much difference between the two places (Table 72). Observed and expected frequency of heterozygote is given in Table 74.

Aldehyde oxidase:

Penaeus indicus

Genes controlling this enzyme was identified in the samples which were collected from four different locations

Fig. 18. Expression of alcohol dehydrogenase in different tissues of Parapenaecopsis stylifera.

Fig. 19. Expression of different genotypes of alcohol dehydrogenase in hepatopancreas tissues of Parapenaecopsis stylifera

Fig. 18. Expression of alcohol dehydrogenase in different tissues of Parapenaeopsis stylifera.

Fig. 19. Expression of different genotypes of alcohol dehydrogenase in hepatopancreas tissues of Parapenaeopsis stylifera

Fig. 18

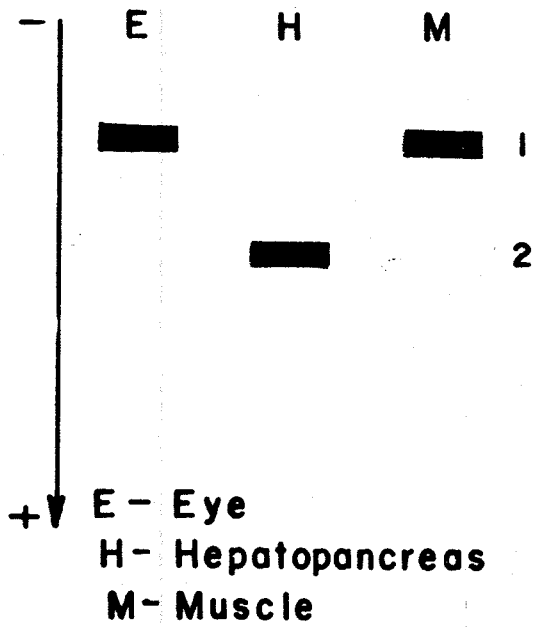


Fig. 19

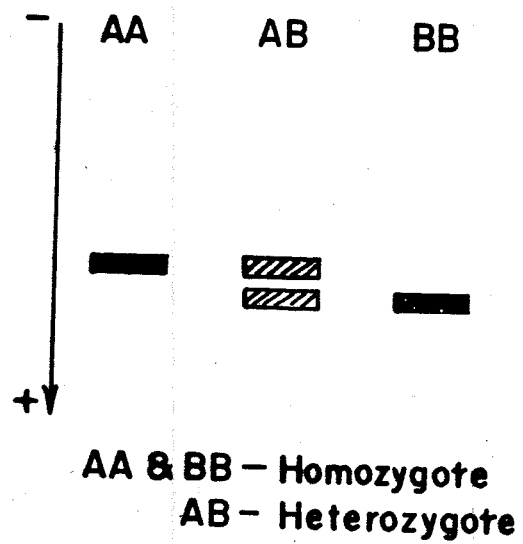


Plate 17: Showing the different genotypes of alcohol dehydrogenase (Adh-2) in hepatopancreas tissue of Parapenaeopsis stylifera AA, BB-homozygotes, AB-heterozygote.

Plate 18: Showing the different genotypes of aldolase (Ald-1) in muscle tissues of Penaeus indicus AA, BB, CC-homozygotes, AB, BC, heterozygotes

viz. Cochin, Tuticorin, Madras and Waltair. Enzymatic expression revealed 3 loci (Fig. 20) viz. 2 loci in hepatopancreas and one in muscle tissue. Ao-1 locus located in slowest migrating zone in hepatopancreas exhibited polymorphism in all the localities (Fig. 21; Plate 19). Allele frequency was calculated and presented in Table 71. Details of observed and expected phenotypes are tabulated (Table 51).

P. stylifera

Aldehydeoxidase enzyme was examined in eye, hepatopancreas and muscle tissues expressed 3 loci giving rise to three bands of aldehydeoxidase activity in the zymogram (Fig. 22). Hepatopancreas expressed 2 loci whereas muscle one locus. Monomeric subunit structure of this enzyme was revealed by their phenotypic expression. The two diffused bands were scored as heterozygote. Since the allele frequency value exceeded 0.95 it was considered to be monomorphic.

Observed allele frequencies were given in Table 72. There is not much difference noticed in these values in the samples of Bombay and Cochin for this enzyme.

Phenotypic distribution according to Ao-2 and Ao-3 is given Table 52.

Table 51: Observed and expected phenotype frequency of aldehyde oxidase (Ao-1) observed in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{\chi^2}$ |
|-----------|----------|------|------|-----|--------------------|
| Cochin | Observed | 20 | 2 | 14 | 30.16 |
| | Expected | 12 | 18 | 6 | |
| Tuticorin | Observed | 20 | 2 | 14 | 30.2 |
| | Expected | 12 | 18 | 6 | |
| Madras | Observed | 22 | 1 | 13 | 32.5 |
| | Expected | 14 | 17 | 5 | |
| Waltair | Observed | 23 | 1 | 12 | 32.1 |
| | Expected | 15.3 | 16.3 | 4.3 | |

Table 52: Observed and expected phenotype frequency of aldehyde oxidase (Ao-2 and Ao-3) observed in Parapenaepsis stylifera with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|----|-----|------|---------------|
| Ao-2 | | | | | |
| Cochin | Observed | 34 | 1 | 1 | 16 |
| | Expected | 33 | 2.9 | 0.06 | |
| Bombay | Observed | 34 | 2 | 0 | 0.035 |
| | Expected | 34 | 1.9 | 0.3 | |
| Ao-3 | | | | | |
| Cochin | Observed | 35 | 0 | 0 | 3.56 |
| | Expected | 34 | 1.9 | 0.3 | |
| Bombay | Observed | 34 | 2 | 9 | 0.305 |
| | Expected | 34 | 1.9 | 0.3 | |

Fig. 20. Expression of aldehyde oxidase in different tissues of Penaeus indicus.

Fig. 21. Expression of different genotypes of aldehydeoxidase (Ao-1) in hepatopancreas tissue of Penaeus indicus.

Fig.20

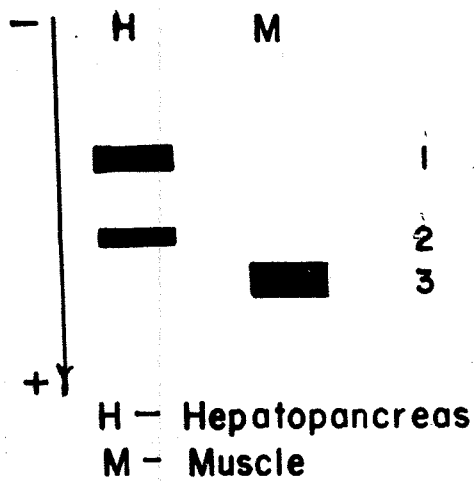


Fig. 21

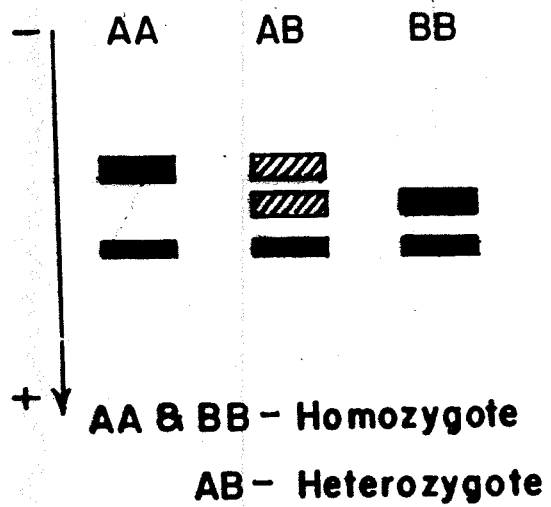


Fig. 22. Expression of aldehyde oxidase in different tissues of Parapenaeopsis stylifera.

Fig. 23. Expression of aldolase in muscle tissue of Penaeus indicus.

Fig. 24. Expression of different genotypes of aldolase (Ald-1) in muscle tissue of Penaeus indicus

Fig.23

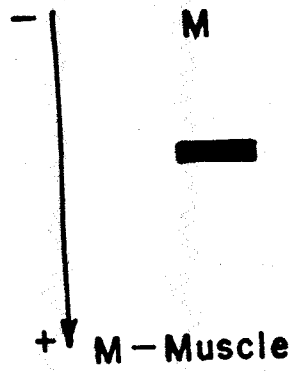


Fig.22

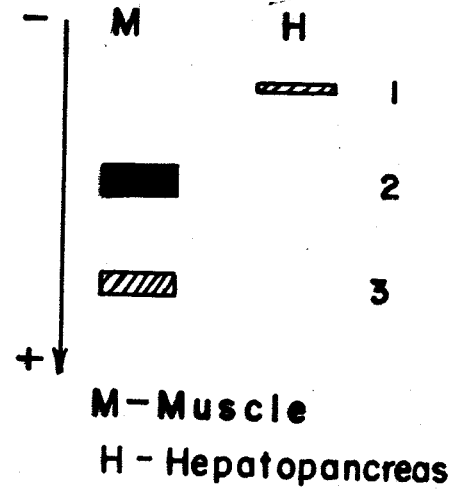
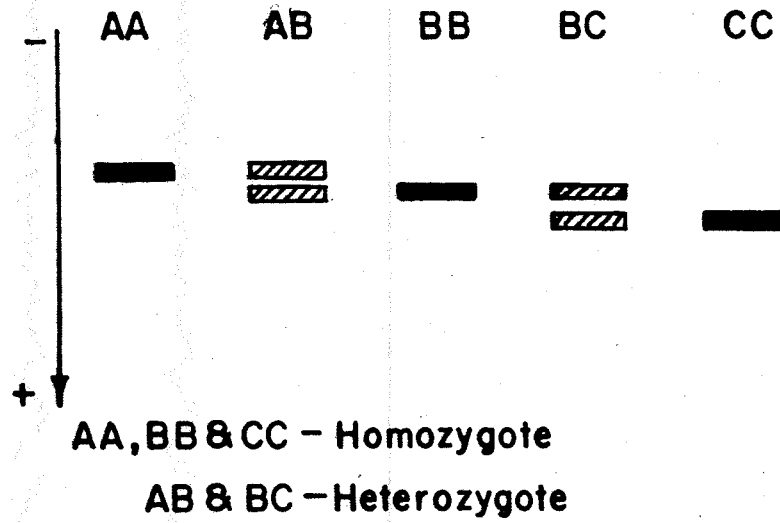
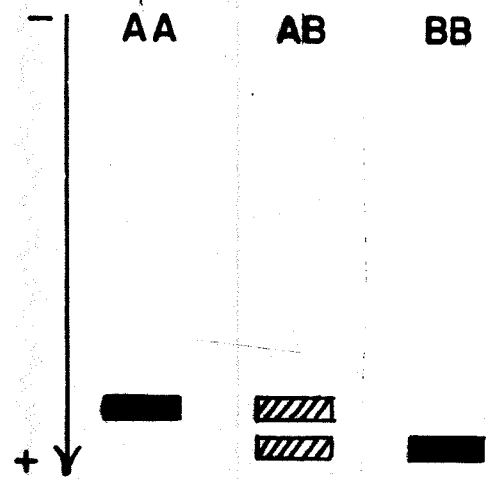


Fig.24



**Fig. 25. Expression of different genotype of
alkaline phosphatase (Alph) in muscle
tissues of Penaeus indicus.**

Fig.25



AA & BB - Homozygote
AB - Heterozygote

Fig. 26. Expression of alkaline phosphatase in different tissues of Parapenaeopsis stylifera.

Fig. 27. Expression of different genotypes of alkaline phosphatase (Alph-2) in muscle tissue of Parapenaeopsis stylifera.

Fig. 26

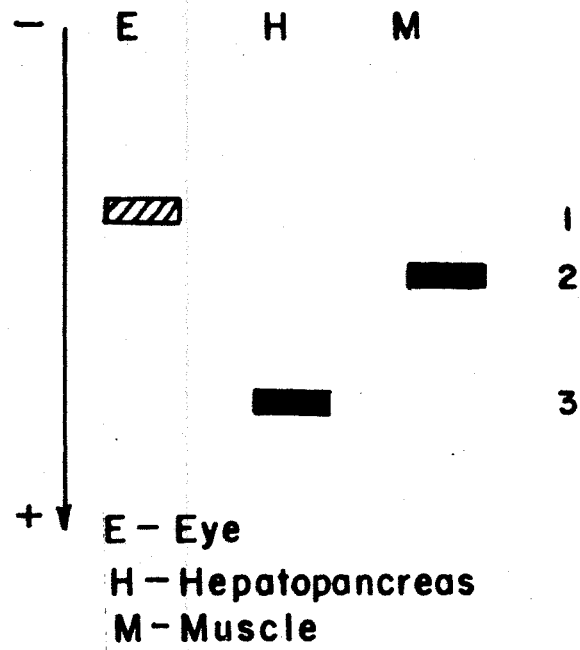


Fig. 27

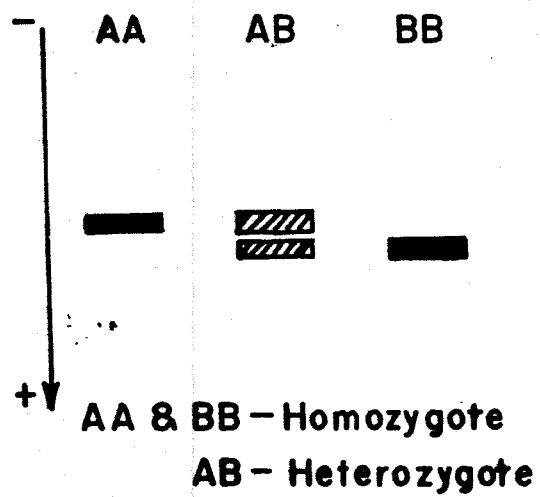


Plate 19: Showing the different genotypes of aldehyde oxidase (Ao-1) in hepatopancreas tissue of Penaeus indicus AA, BB-homozygotes, BB-heterozygote.

Plate 20: Showing the different genotypes of alkaline phosphatase (Alph-2) in muscle tissues of Parapenaeopsis stylifera AA, BB-homozygotes AB-heterozygote.

Aldolase:

P. indicus

Aldolase enzyme pattern was analysed in eye, hepatopancreas and muscle of P. indicus collected from different localities like Cochin, Tuticorin, Madras and Waltair. Muscle tissue showed a single zone of enzyme activity (Fig. 23). Polymorphism was observed in all locations with 3 alleles (Fig. 24; Plate 18).

Allele frequency observed is given in Table 71. Details of phenotypes observed is tabulated (Table 53).

Alkaline phosphatase:

P. indicus

This enzyme was analysed for genetic variation studies in muscle tissue. Samples were collected only from Waltair. It showed polymorphism in muscle tissue. Phenotypic expression showed a 2 banded heterozygote revealing it to be having monomeric subunit structure (Fig. 25). Number of phenotypes observed is give in table 54.

P. stylifera

Phenotypic expression of alkaline phosphatase enzyme in different tissue were found out. Eye hepatopancreas and muscle showed single zone of enzyme activity in different locations. Thus 3 locus were observed for this enzyme (Fig. 26).

Table 53: Observed and expected phenotype frequency of aldolase (Ald-1) observed in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | BC | CC | AC | χ^2 |
|-----------|----------|-----|------|------|------|-----|-----|----------|
| Cochin | Observed | 5 | 1 | 13 | 2 | 15 | 0 | 56.59 |
| | Expected | .8 | 4.3 | 5.4 | 12.8 | 7.6 | 5 | |
| Tuticorin | Observed | 6 | 2 | 19 | 1 | 8 | 0 | 49.994 |
| | Expected | 1.4 | 7.6 | 10.6 | 10.3 | 2.5 | 3.6 | |
| Madras | Observed | 6 | 1 | 20 | 2 | 7 | 0 | 51.08 |
| | Expected | 1.2 | 7.5 | 12.2 | 9.9 | 2 | 3.1 | |
| Waltair | Observed | 4 | 0 | 27 | 2 | 3 | 0 | 60.512 |
| | Expected | 0.4 | 6.22 | 21.8 | 6.2 | .4 | .9 | |

Table 54: Observed and expected phenotype frequency of alkaline phosphatase (Alph) observed in Penaeus indicus.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|------|----|----|---------------|
| Waltair | Observed | 29 | 2 | 5 | 23.07 |
| | Expected | 24.9 | 10 | 1 | |

Table 55: Observed and expected phenotype frequency of alkaline phosphatase (Alph-2) observed in Parapenaeopsis stylifera with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|------|-----|-----|---------------|
| Cochin | Observed | 32 | 2 | 2 | 14.58 |
| | Expected | 30 | 5.5 | .25 | |
| Bombay | Observed | 31 | 2 | 3 | 20.84 |
| | Expected | 28.4 | 7.1 | .4 | |

Muscle was tested for genetic variation. Heterozygote showed 2 bands indicating monomeric structure of the enzyme. Alph-2 locus analysed showed allelic variants (Fig.27; Plate 20), in both location. Phenotypic expressions in this locus is given in Table 55. Allele frequency observed in given in Table 72.

Esterase:

P. indicus

Tissue expression of esterase was observed in eye hepatopancreas and muscle. The pattern observed for this enzyme was not consistent and therefore genetic variation study couldn't be carried out.

P. stylifera

P. stylifera exhibited polymorphism in esterase enzyme collected from Cochin and Bombay. It expressed two zones of activity in hepatopancreas, one zone of enzyme activity in muscle tissue and three zones of activity in eye tissue (Fig. 28). Genetic analysis was carried out in eye tissue. Phenotypic variation was observed in eye at the Est-2 locus (Fig. 29; Plate 21).

Observed and expected phenotypic frequency was tabulated (Table 56). Allele frequency is given in Table 72.

Muscle was tested for genetic variation. Heterozygote showed 2 bands indicating monomeric structure of the enzyme. Alph-2 locus analysed showed allelic variants (Fig.27; Plate 20). in both location. Phenotypic expressions in this locus is given in Table 55. Allele frequency observed in given in Table 72.

Esterase:

P. indicus

Tissue expression of esterase was observed in eye hepatopaneas and muscle. The pattern observed for this enzyme was not consistent and therefore genetic variation study couldn't be carried out.

P. stylifera

P. stylifera exhibited polymorphism in esterase enzyme collected from Cochin and Bombay. It expressed two zones of activity in hepatopaneas, one zone of enzyme activity in muscle tissue and three zones of activity in eye tissue (Fig. 28). Genetic analysis was carried out in eye tissue. Phenotypic variation was observed in eye at the Est-2 locus (Fig. 29; Plate 21).

Observed and expected phenotypic frequency was tabulated (Table 56). Allele frequency is given in Table 72.

Table 56: Observed and expected phenotype frequency of esterase (Est-2) seen in Parapenseopsis stylifera with Chi-square

| Location | | AA | BB | BB | $\frac{2}{Z}$ |
|----------|----------|------|-----|-----|---------------|
| Cochin | Observed | 30 | 3 | 3 | 13.8 |
| | Expected | 27.6 | 7.9 | .56 | |
| Bombay | Observed | 33 | 1 | 2 | 19.2 |
| | Expected | 31.2 | 4.7 | .2 | |

Table 57: Observed and expected phenotype frequency of alpha glycerophosphate (Gpdh-1) observed in Parapenseopsis stylifera with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|----|-----|------|---------------|
| Cochin | Observed | 33 | 3 | 0 | 0.063 |
| | Expected | 30 | 2.9 | 0.06 | |
| Bombay | Observed | 34 | 1 | 1 | 16 |
| | Expected | 33 | 2.9 | 0.06 | |

Fig. 28. Expression of esterase in different tissues of Parapenseopsis stylifera

Fig. 29. Expression of different genotypes of esterase (Est-2) in eye tissue of Parapenseopsis stylifera.

Fig. 28

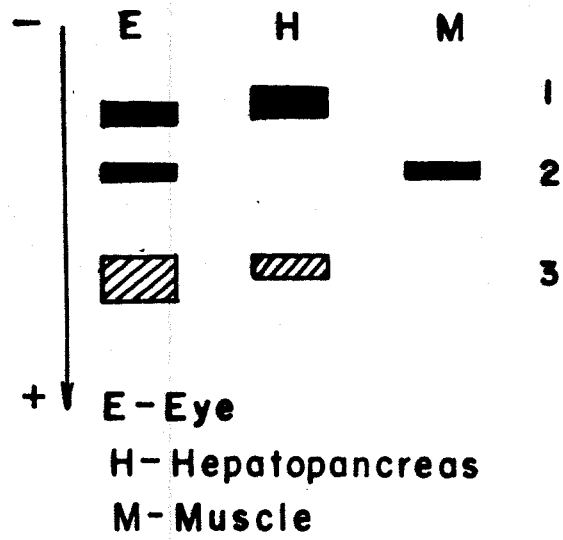


Fig. 29

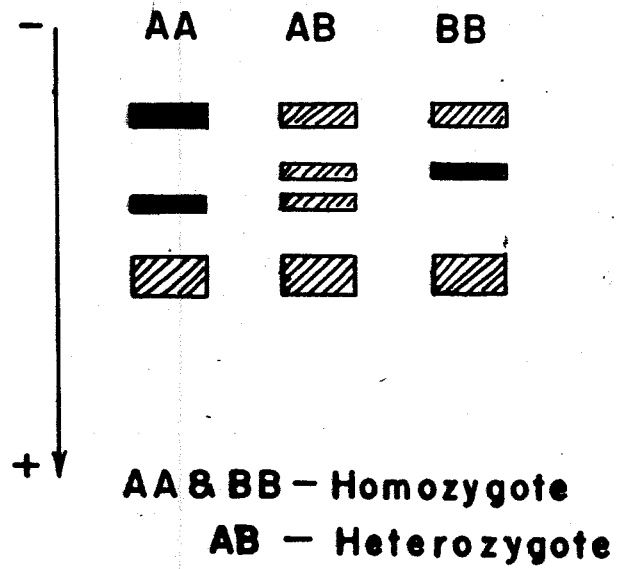


Fig. 30. Expression of alphaglycerophosphate dehydrogenase in hepatopancreas tissue of Penaeus indicus and Parapenaeopsis stylifera.

Fig. 31. Expression of lactate dehydrogenase in different tissues of Penaeus indicus

Fig.30

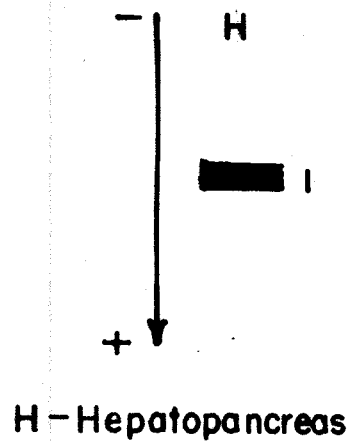
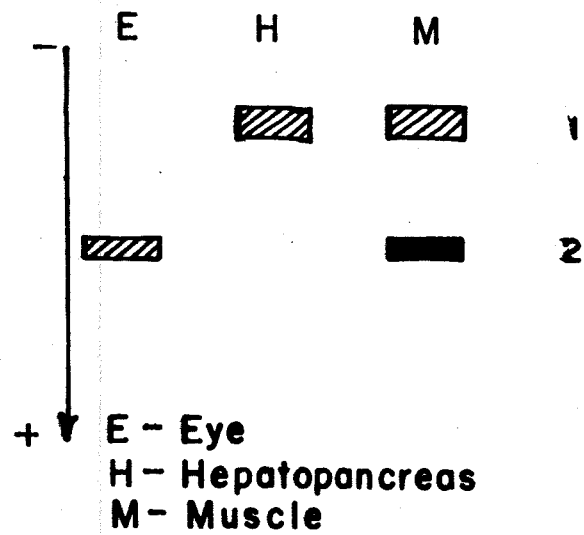


Fig.31



**Fig. 32. Expression of malate dehydrogenase in
different tissues of Penaeus indicus.**

**Fig. 33. Expression of different genotypes of
malate dehydrogenase (Mdh-1) in eye
tissue of Penaeus indicus.**

Fig.32

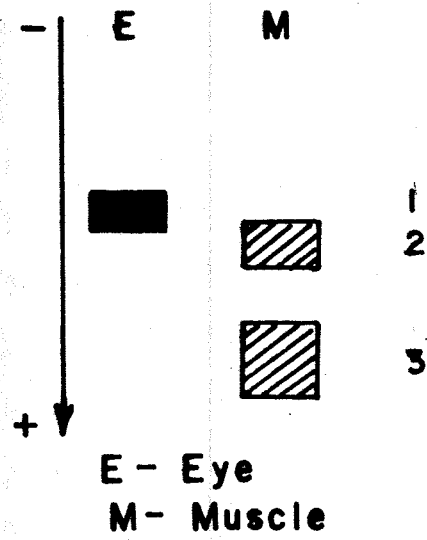


Fig.33

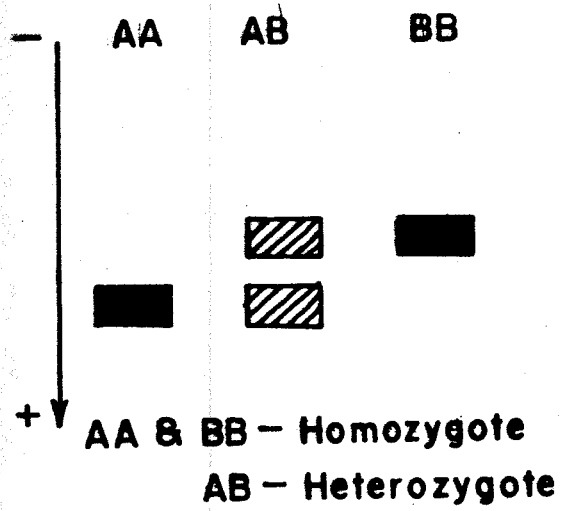


Plate 21: Showing the different genotypes of esterase
(Est-2) in eye tissues of Parapenaecopsis
stylifera AA, BB-homozygotes, AB-heterozygote

Plate 22: Showing the different genotypes of malate
dehydrogenase (Mdh-1) in eye tissue of
Penaeus indicus AA, BB-homozygotes,
AB-heterozygote.

Alpha glycerophosphate dehydrogenase:

P. indicus and P. stylifera showed activity for this enzyme only in hepatopancreas tissue (Fig. 30). Allele frequency was calculated for the observed allelic variants. Since the observed allele frequency of the common allele exceeded 0.95, it was taken as monomorphic loci in both species.

The allele frequency is given in Table 72. The observed phenotypic distribution for P. stylifera is given in Table 57.

Lactate dehydrogenase:

P. indicus

Two zones of lactate dehydrogenase activity were observed. Eye and hepatopancreas expressed one band each having different mobility. These two bands were also observed in muscle tissue (Fig. 31). Since consistent pattern couldn't be observed, these were not utilized for further electrophoretic analysis work.

Malate dehydrogenase:

P. indicus

Malate dehydrogenase resolved as 3 bands of activity in eye and muscle (Fig. 32). Malate dehydrogenase expressed

Table 58: Observed and expected phenotype frequency of malate dehydrogenase (Mdh-1) observed in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|-----------|----------|-----|------|------|---------------|
| Cochin | Observed | 12 | 1 | 23 | 32.1 |
| | Expected | 4.3 | 16.3 | 15.3 | |
| Tuticorin | Observed | 13 | 1 | 22 | 32.4 |
| | Expected | 5 | 16.9 | 14 | |
| Madras | Observed | 10 | 2 | 24 | 27 |
| | Expected | 3.4 | 15.3 | 17.4 | |
| Waltair | Observed | 12 | 1 | 23 | 32.1 |
| | Expected | 4.3 | 16.3 | 15.3 | |

Table 59: Observed and expected phenotype frequency of malate dehydrogenase (Mdh-1) observed in Parapenaeopsis stylifera with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|------|-----|-----|---------------|
| Cochin | Observed | 33 | 1 | 2 | 22.6 |
| | Expected | 31 | 4.6 | .17 | |
| Bombay | Observed | 30 | 2 | 4 | 21.3 |
| | Expected | 26.7 | 8.6 | .69 | |

monomeric pattern by its 2 banded heterozygote nature in eye. Mdh-1 analysed in eye tissue showed allelic variants (Fig. 33; Plate 22) and showed polymorphism in all the localities sampled. Observed and expected phenotypic frequency is tabulated (Table 58). Allele frequency calculated is given in Table 71.

P. stylifera

P. stylifera expressed two bands of enzyme activity like P. indicus in eyes and muscle tissue. Mdh-1 locus was found to be polymorphic in all the localities (Fig. 34). Two banded heterozygotic nature revealed its monomeric form with 2 alleles (Fig. 35; Plate 23). Phenotypic expression observed in Cochin and Bombay is given in Table 59.

The allele frequency observed is presented in Table 59. Observed and expected phenotype frequency is tabulated (Table 59).

Malic enzyme

P. indicus

Malic enzyme expressed one loci in eyes and another in muscle (Fig. 36). Me-1 found in eyes expressed polymorphism with 2 alleles. Two banded heterozygote was seen (Fig. 37; Plate 24). Phenotypes observed is given in Table 60.

Fig. 34. Expression of malate dehydrogenase in different tissues of Parapenaeopsis stylifera.

Fig. 35. Expression of different genotypes of malate dehydrogenase (Mdh-1) in eye tissue of Parapenaeopsis stylifera.

Fig. 34

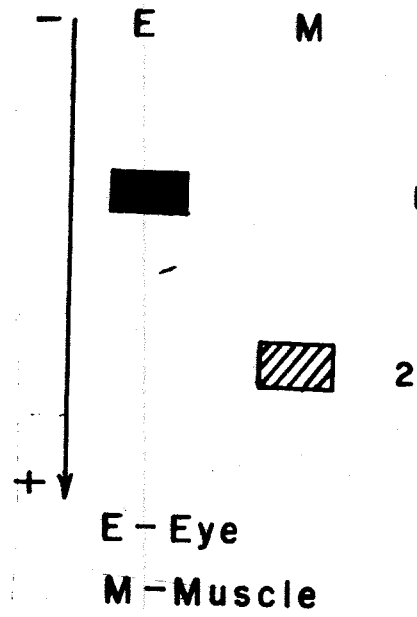


Fig.35

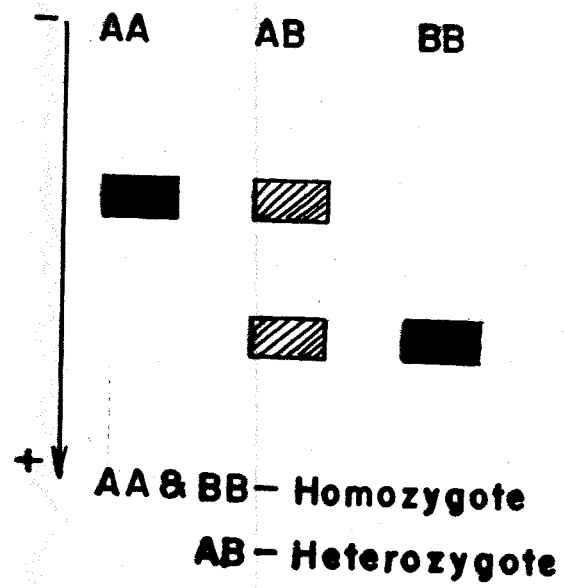


Fig. 36. Expression of malic enzyme in different tissues of Penaeus indicus

Fig. 37. Expression of different genotypes of malic enzyme (Me-1) in eye tissue of Penaeus indicus.

Fig.36

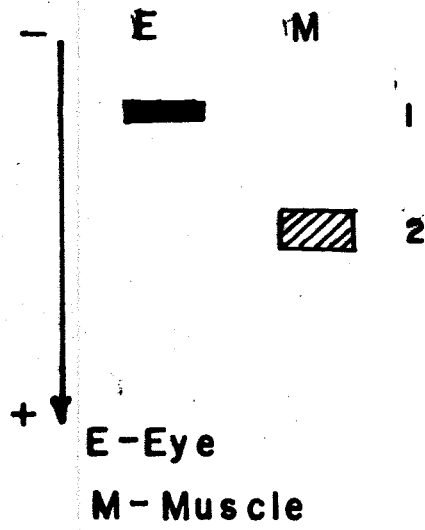
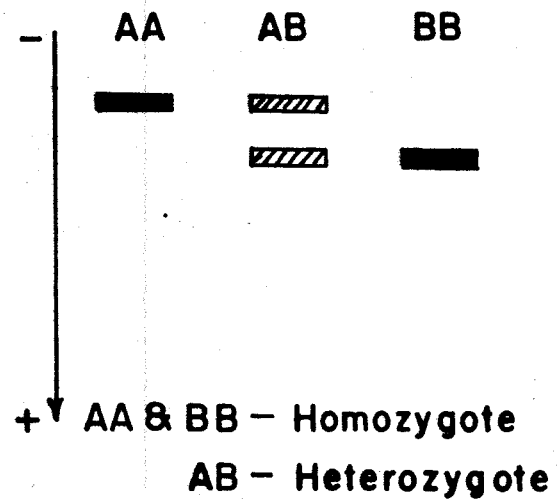


Fig. 37



Allele frequency obtained is given in Table 71. There is slight enhancement in the allele frequency of the allele A from Cochin, Tuticorin, Madras and Waltair.

Observed and expected phenotype frequency is tabulated (Table 60).

P. stylifera

Electrophoretic analysis revealed one locus each in eye and muscle (Fig. 38). Me-1 in eye was found to show polymorphism at Cochin. Phenotypic expression seen is given in Table 61.

Octanol dehydrogenase:

P. indicus

The enzyme expressed zones of enzyme activity in different tissues. The loci observed were Odh-2 in eye, Odh-1 in hepatopancreas and Odh-3 in muscle (Fig.39). The Odh-2 locus showed polymorphism only at Waltair (Fig. 40; Plate 25). Phenotypic expressions with one and 3 banded patterns in homozygous and heterozygous nature, respectively, suggested a dimeric polypeptide structure for Octanol dehydrogenase enzyme. Different phenotypic expression observed is given in Table 62.

Table 60: Observed and expected phenotype frequency in malic enzyme (Me-1) seen in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|-----------|----------|------|-----|-----|---------------|
| Cochin | Observed | 12 | 0 | 6 | 18 |
| | Expected | 8 | 8 | 2 | |
| Tuticorin | Observed | 13 | 0 | 5 | 17.9 |
| | Expected | 9.4 | 7.2 | 1.4 | |
| Madras | Observed | 14 | 1 | 4 | 17.8 |
| | Expected | 10.9 | 6.2 | 0.9 | |
| Waltair | Observed | 14 | 1 | 3 | 11.8 |
| | Expected | 11.7 | 5.6 | .7 | |

Table 61: Observed and expected phenotype frequency of malic enzyme (Me-1) observed in Parapenaeopsis stylifera with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|------|------|-----|---------------|
| Cochin | Observed | 20 | 4 | 12 | 21.4 |
| | Expected | 13.4 | 17.4 | 5.4 | |

Table 62: Observed and expected phenotype frequency of octanol dehydrogenase (Odh-2) observed in Penaeus indicus with Chi-square value.

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|-----------|----------|-----|------|-------|---------------|
| Cochin | Observed | 0 | 0 | 36 | |
| | Expected | 0 | 0 | 36 | |
| Tuticorin | Observed | 0 | 0 | 36 | |
| | Expected | 0 | 0 | 36 | |
| Madras | Observed | 0 | 0 | 36 | |
| | Expected | 0 | 0 | 36 | |
| Waltair | Observed | 2 | 2 | 32 | 14.6 |
| | Expected | .24 | .549 | 30.25 | |

Table 63: Observed and expected phenotype frequency of octanol dehydrogenase (Odh-2) observed in Parapenaeopsis stylifera with Chi-square value

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|----|------|------|---------------|
| Cochin | Observed | 34 | 2 | 0 | 0.0252 |
| | Expected | 34 | 1.9 | 0.02 | |
| Bombay | Observed | 35 | 1 | 0 | 0.0609 |
| | Expected | 35 | 0.97 | 0.06 | |

Fig. 38. Expression of malic enzyme in different tissues of Parapenaeopsis stylifera.

Fig. 39. Expression of Octanol dehydrogenase in different tissues of Penaeus indicus.

Fig. 40. Expression of different genotypes of octanol dehydrogenase (Odh-2) in eye tissue of Penaeus indicus.

Fig. 38

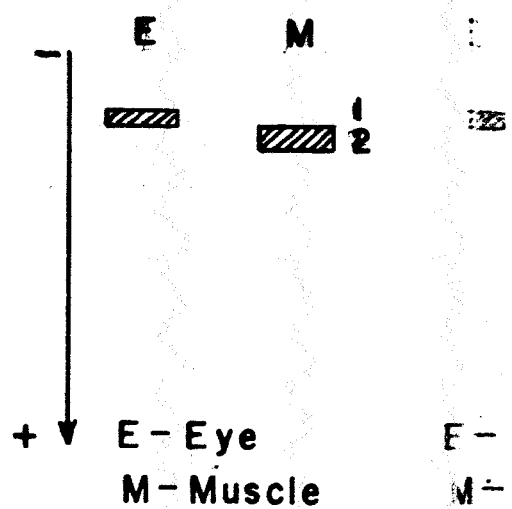


Fig. 39

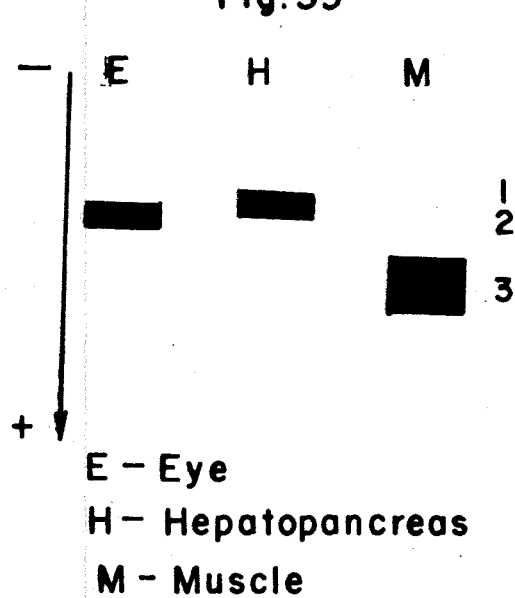


Fig. 40

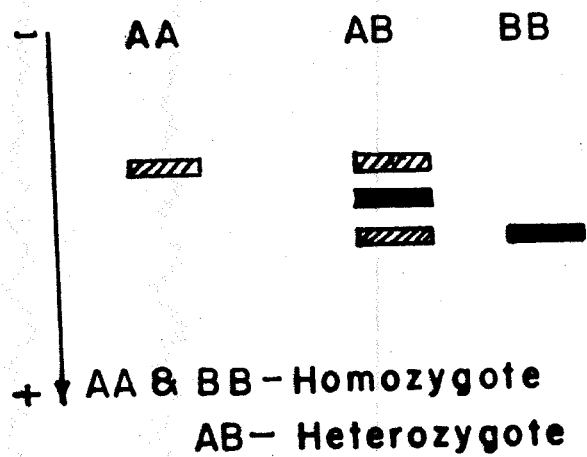


Fig. 41. Expression of octanol dehydrogenase in different tissues of Parapenaeopsis stylifera.

Fig. 42. Expression of 6-Phosphogluconate dehydrogenase observed in difference tissues of Penaeus indicus.

Fig. 41

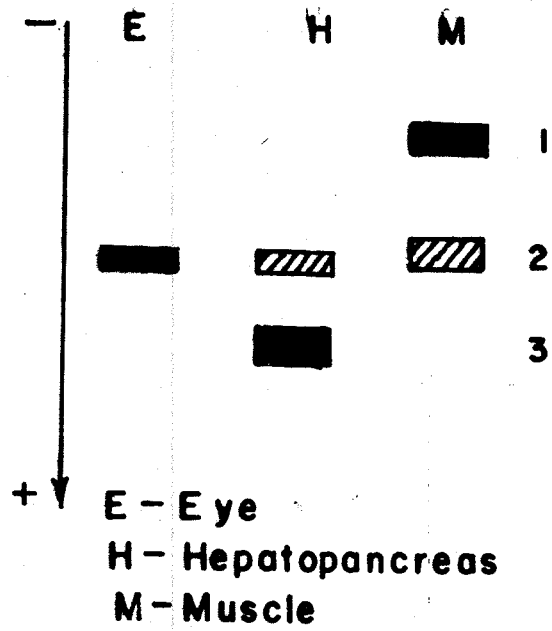


Fig. 42

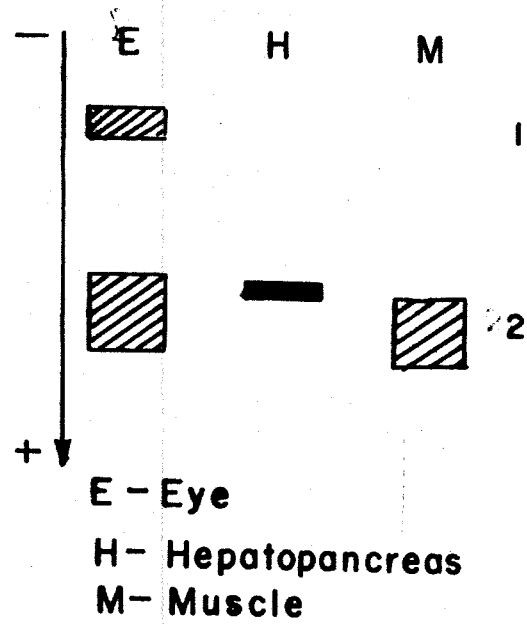
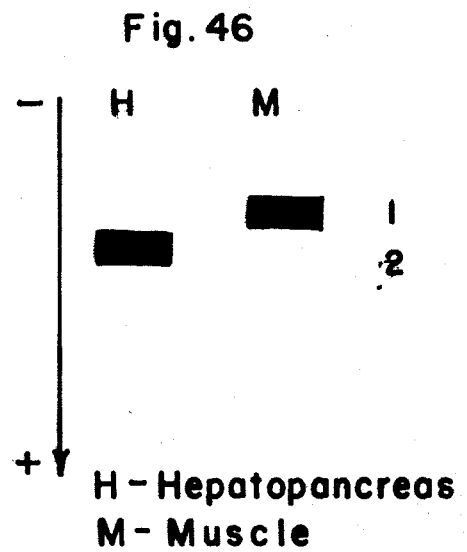
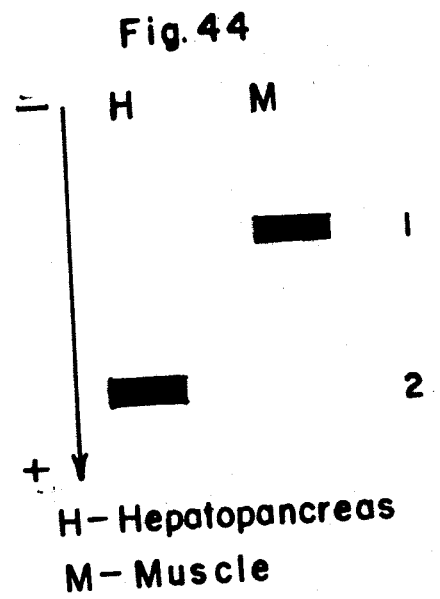
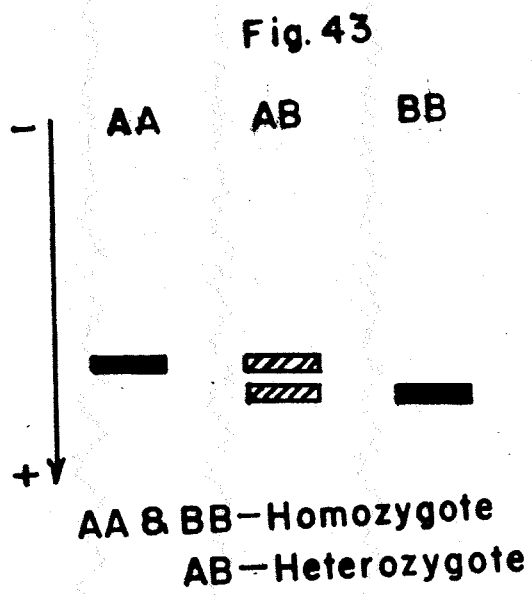


Fig. 43. Expression of different genotypes of 6-phosphogluconate dehydrogenase (6-Pgdh-2) observed in hepatopancreas tissue of Penaeus indicus.

Fig. 44. Expression of 1-pyrroline dehydrogenase in different tissues of Penaeus indicus.

Fig. 45. Expression of 1-pyrroline dehydrogenase in different tissue of Parapenaeopsis stylifera.

Fig. 46. Expression of sorbitol dehydrogenase in different tissue of Penaeus indicus.



This showed 3 phenotypes only in Waltair population. Alleles frequency is given in Table 71.

P. stylifera

P. stylifera expressed 3 loci in its tissue one locus in eye (odh-2), 2 loci in hepatopaneas (odh-2 and odh-3) and in muscle (Odh-1 and odh-2) (Fig.41). Phenotypic expression observed in odh-2 is given in Table 63. As the allele frequency was above 0.95 it was considered as monomorphic.

Allele frequency found is given in Table 72.

6-Phosphogluconate dehydrogenase:

P. indicus

There are two loci for 6-Phosphogluconate dehydrogenase giving rise to a fast 6-Pgdh-1 and slow 6-Pgdh-2 bands of activity. Fast locus was observed in eye, hepatopaneas and muscle tissues whereas slow locus was expressed only in eye (Fig.42). This was analysed in hepatopaneas and muscle. Heterozygote showed 2 banded phenotype and homozygote with single banded phenotype in hepatopaneas indicating a typical pattern of monomer (Fig. 43; Plate 26). 6 Pgdh-2 expressed polymorphism with 2 alleles in all the localities. Details of phenotypic expression observed are given in Table 64.

Allele frequencies observed are given in Table 71.

Table 64: Observed and expected phenotype frequency of 6-phospho-gluconate dehydrogenase (6-Pgdh-2) observed in Penaeus indicus with Chi-square value

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|-----------|----------|-----|------|-------|---------------|
| Cochin | Observed | 15 | 1 | 20 | 31.9 |
| | Expected | 6.7 | 17.6 | 1.67 | |
| Tuticorin | Observed | 15 | 1 | 20 | 31.9 |
| | Expected | 6.7 | 17.6 | 11.67 | |
| Madras | Observed | 14 | 1 | 21 | 32.3 |
| | Expected | 5.8 | 17.3 | 12.8 | |
| Waltair | Observed | 15 | 2 | 19 | 28.4 |
| | Expected | 7.1 | 17.8 | 11.1 | |

Table 65: Observed and expected Phenotype frequency of 1-Pyrroline dehydrogenase (1-Pydh-1) observed in Parapenaeopsis stylifera with Chi-square value

| Location | | AA | AB | BB | $\frac{2}{Z}$ |
|----------|----------|----|-----|------|---------------|
| Cochin | Observed | 34 | 1 | 1 | 15.93 |
| | Expected | 33 | 2.8 | 0.06 | |
| Bombay | Observed | 35 | 1 | 0 | 0.009 |
| | Expected | 35 | .95 | .006 | |

1-Pyrroline dehydrogenase:

P. indicus

This enzyme expressed 2 zones of activity, one in hepatopancreas (1-Pydh-2) and another in muscle tissue (1-Pydh-1) (Fig. 44). But it didn't express any allelic variation and hence this was considered as monomorphic locus.

P. stylifera

Hepatopancreas and muscle expressed the activity for this enzyme (Fig. 45). Since the expression was consistent, it was analysed for genetic variation studied in hepatopancreas and the frequency analysis proved it to be monomorphic.

Observed and expected phenotype frequency is tabulated (Table 65).

Sorbitol dehydrogenase

P. indicus

This enzyme showed 2 bands of activity; one in hepatopancreas and another in muscle (Fig. 46). Phenotypic expression studied in muscle tissue revealed only one allele from these loci. So it was suggested to be monomorphic.

Tetrazolium oxidase:

P. indicus

P. indicus exhibited zones of enzyme expression in three

tissues; To-1 and To-2 loci are expressed in eye, To-3 and To-4 loci in hepatopancreas and To-2 locus in muscle tissue (Fig. 47).

As allelic frequencies analysed in muscle was found to be above 0.95 the locus was taken as monomorphic.

P. stylifera

This enzyme exhibited 2 zones of activity in P. stylifera (one locus in eye, 2 loci in hepatopancreas and one in muscle tissue)(Fig.48). To-1 in hepatopancreas showed allelic variant with a monomer pattern. To-2 (Fig.49) Plate 27) expressed low level of allele frequency so it is discarded from the further calculation. Details of phenotypes observed in To-1 are shown in Table No.66.

Allele frequency calculated is given in Table 72. There is not much variation observed in allele frequencies of these 2 places.

Hardy-Weinberg equilibrium

Details of expected Hardy-weinberg equilibrium distribution of phenotypes analysed are shown (Table Nos.48-66). Difference between observed and expected distribution was tested and found to be significant in Acph-3, Ao-1, Ald-1, Alph, Mdh-1, Me-1, Odh-2 and 6-Pgdh-2 for P. indicus and

Table 66: Observed and expected phenotype frequency observed in Parapeneopsis stylifera with Chi-square value.

| Location | | AA | AB | BB | χ^2 |
|----------|----------|-------|------|------|----------|
| To-1 | | | | | |
| Cochin | Observed | 20 | 2 | 14 | 28.21 |
| | Expected | 12.25 | 17.5 | 6.25 | |
| Bombay | Observed | 18 | 1 | 17 | 32.1 |
| | Expected | 9.15 | 18 | 8.5 | |
| To-2 | | CC | CD | DD | 2 |
| Cochin | Observed | 33 | 3 | 0 | 0.063 |
| | Expected | 33 | 2.9 | 0.06 | |
| Bombay | Observed | 33 | 3 | 0 | 0.063 |
| | Expected | 33 | 2.9 | 0.06 | |

**Fig. 47. Expression of tetrazolium oxidase in
different tissues of Penaeus indicus.**

**Fig. 48. Expression of tetrazolium oxidase in
different tissues of Parapenaeopsis stylifera**

Fig. 47

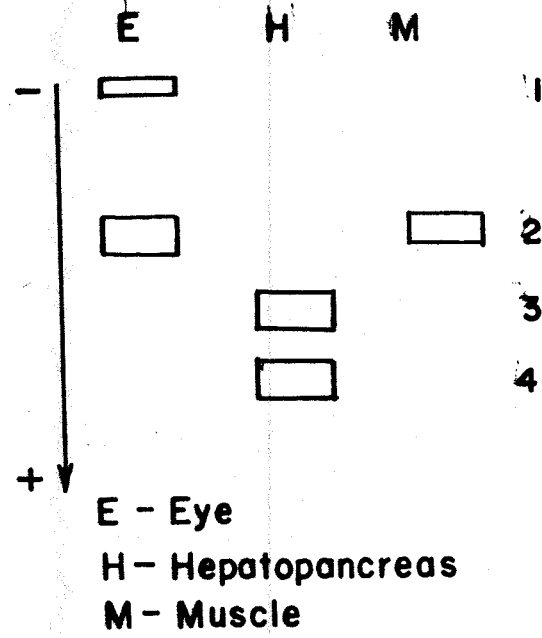


Fig. 48

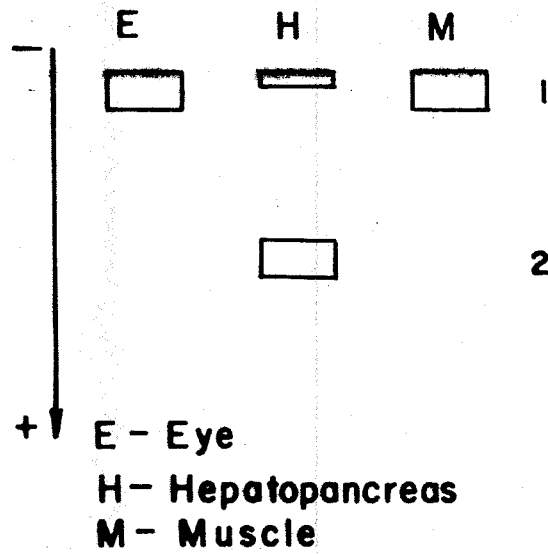


Fig. 49. Expression of different genotypes of
tetrazolium oxidase (To-2) in hepato-
pancreas tissue of Parapenaeopsis
stylifera.

Fig. 49

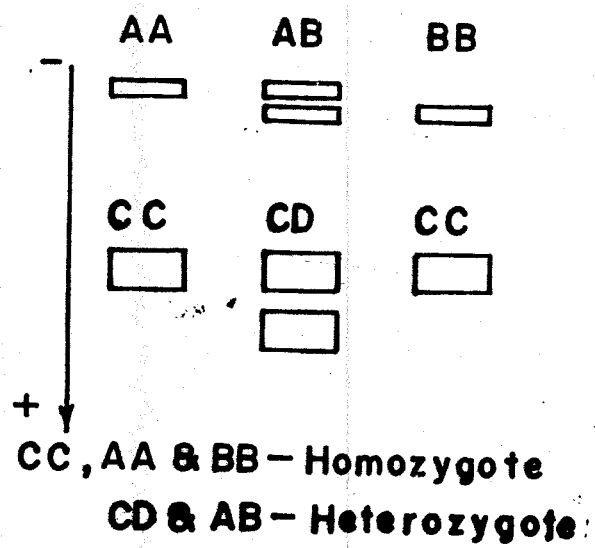


Plate 27: Showing the different genotypes of tetrazolium oxidase (To-2) in hepatopancreas tissue of Parapenaeopsis stylifera AA, BB-homozygotes, AB-heterozygote.

AA AA BB AB AB

To₁

To₂



also for Acph-2, Adh-2, Ao-2 from Cochin, Alph-2, Est-2, Gpdh-1 from Bombay, Mdh-1, Me-1, 1-Pydh-1 in Cochin and To-1 in P. stylifera and insignificant in Ao-2 of Bombay, Gpdh-1 of Cochin, Odh-2, Pydh-1 from Bombay and To-2 in P. stylifera. Significant values were produced by deficiency of heterozygotes/excess of homozygotes.

Heterozygosity analysis was carried out to find out the amount of genetic variation in the population. So, observed and expected frequency of heterozygotes in these populations was calculated. The difference arose between these two was tabulated for statistical significance as seen in Acph-3, Ald-1, Ao-1, Alph-1, Mdh-1, Me-1, Odh-2 and 6-Pgdh in P. indicus, Mdh-1, Me-1 and To-1 in P. stylifera and insignificant in To-2, Ao-2, Odh-2 and Gpdh and Pydh for P. stylifera. Significant values obtained in polymorphic loci were due to the excess of homozygotes/deficiency of heterozygotes in these two prawn species.

Genetic variation within prawn populations

Overall estimate of average frequency of heterozygotes per locus \bar{H} in a population was calculated by averaging observed frequency of heterozygote (H_o) overall loci sampled. The average frequency of heterozygotes per locus (\bar{H}) for P. indicus in Cochin was 0.0114 ± 0.02 , in Tuticorin 0.0114 ± 0.02 ,

in Madras 0.0096 ± 0.02 and in Waltair 0.0142 ± 0.02 . Total estimate of average frequency of heterozygotes per locus H is 0.0105 ± 0.02 . The same estimate was calculated for P. stylifera collected from Cochin and Bombay. The average frequency of heterozygotes per locus H for P. stylifera collected in Cochin is 0.3 ± 0.03 and for Bombay it is 0.025 ± 0.03 . Total average frequency of heterozygote per locus H was found out to be $.026 \pm 0.03$ for P. stylifera.

Proportion of polymorphic loci 'p' was calculated for P. stylifera in Cochin and it was found out to be 0.304 and for P. stylifera in Bombay it was 0.273. For P. indicus, the proportion of polymorphic loci was found out to be 0.261 in Cochin, Tuticorin and Madras and 0.333 in Waltair. Average proportion of polymorphic loci per population P_p is 0.2885 for P. stylifera and 0.279 for P. indicus (Table 69 & 70).

Mean number of alleles per locus (A) seen in each population was found out. For P. indicus in Cochin, Tuticorin and Madras it was 1.304 and 1.375 in Waltair. For P. stylifera, mean number of alleles per locus was 1.545 in Cochin and 1.575 in Bombay. For these populations in total, the mean number of alleles per locus was found out to be 1.558 for P. stylifera and 1.322 for P. indicus (Table 75 & 76).

Table 67: Nei's D-genetic distance (above the Diagonal)
I-Genetic identity (below the diagonal)
J(X)-Average homozygosity (on the diagonal)
for P. stylifera and P. indicus

P. stylifera

| Location* | Geographic populations* | |
|-----------|-------------------------|--------|
| | Co | Bom. |
| Co | (.887) | .009 |
| Bom | .991 | (.878) |

P. indicus

| Location ^a | Geographic populations ^a | | | |
|-----------------------|-------------------------------------|--------|--------|-------|
| | Co | Tun | Mds | Wal. |
| Co | (.827) | .002 | .003 | .055 |
| Tun | .998 | (.828) | .001 | .049 |
| Mds. | .997 | .999 | (.836) | .047 |
| Wal | .947 | .952 | .954 | (.84) |

* Co-Cochin; Bom- Bombay.

a Co-Cochin; Tun-Tuticorin; Mds-Madras; Wal-Waltair

Table 68: Roger's 'D' (Distance) is above diagonal
'S' (Similarity) is below the diagonal for
Penaeus indicus and Parapenaeopsis stylifera.

Penaeus indicus

| Location ^a | Geographic population ^a | | | |
|-----------------------|------------------------------------|-------|-------|-------|
| | Co | Tun | Mds | Wal |
| Co | - | 0.013 | 0.022 | 0.071 |
| Tun | .987 | - | .014 | 0.064 |
| Mds | .978 | .986 | - | 0.056 |
| Wal | .929 | .936 | .944 | - |

Parapenaeopsis stylifera

| Location [*] | Geographic population [*] | |
|-----------------------|------------------------------------|------|
| | Co | Bom |
| Co | -- | 0.03 |
| Bom | .97 | - |

^aCo-Cochin; Tun-Tuticorin; Mds-Madras; Wal-Waltair

^{*}Co-Cochin; Bom-Bombay

Table 69: Details of genetic analysis carried out in different enzymes in different tissues of Penaeus indicus.

| | | | |
|--------------------------------------|----------------------|-------------------------------|----------------|
| 1. Acid phosphatase | Acph-1 x Acph-2 x | Acph-1 - 2 - 4 - 5 - | Acph-3 x + |
| 2. Alcohol dehydrogenase | Adh-2 - 3 - | Adh-1 x 2 x | Adh-2 x |
| 3. Aldehyde oxidase | - | Ao-1 + 2 x | Ao-3 - |
| 4. Aldolase | - | - | Ald-1 x + |
| 5. Alkaline phosphatase | - | - | Alph x + |
| 6. Glycerophosphate dehydrogenase | - | Gpdh-1 x | |
| 7. Lactate dehydrogenase | Ldh-2 - | Ldh-1 - | Ldh-1 - 2 - |
| 8. Malate dehydrogenase | Mdh-1 x + | - | Mdh-2 x 3 x |
| 9. Malic acid | Me-1 x + | - | Me-2 x |
| 10. Octanol dehydrogenase | Odh-2 x + | Odh-1 x | Odh-3 x |
| 11. 6-Phosphogluconate dehydrogenase | Pgdh-1 - 2 - | Pgdh-2 x + | Pgdh-2 x |
| 12. 1-Pyrroline dehydrogenase | - | Pydh-2 x | Pydh-1 x |
| 13. Sorbitol dehydrogenase | - | Sdh-2 - | Sdh-1 x |
| 14. Tetrasolium oxidase | To-1 - 2 - | To-3 - 4 - | To-2 x |

Analysed x
Polymorphic +
Not analysed -

Table 70: Details of genetic analysis carried out in different enzymes in different tissues of Parapanaeopsis stylifera.

| | | | |
|-----------------------------------|---------------------|---------------|--------------|
| 1. Acid phosphatase | Acph-1x | Acph-3- 4- | Acph-2 x+ |
| 2. Alcoholdehydrogenase | Adh-1x | Adh-2 x+ | Adh-1- |
| 3. Aldehyde oxidase | - | Ao-2x 3x | Ao-1x |
| 4. Alkaline phosphatase | Alph-1- | Alph-3- | Alph-2x+ |
| 5. Esterase | Est-1x 2x+ 3x | Est-1- 3- | Est-2- |
| 6. Glycerophosphate dehydrogenase | - | Gpdh-1x | - |
| 7. Malate dehydrogenase | Mdh-1x+ | - | Mdh-2x |
| 8. Malic acid | Me-1x | - | Me-2 |
| 9. Octanol dehydrogenase | Odh-2x | Odh-2x 3x | Odh-1 2x |
| 10. Tetrazolium oxidase | To-1- | To-1+ 2x | To-1x |
| 11. 1-Pyrroline dehydrogenase | | Pydh-1 x | Pydh-1- |

Analysed x
Polymorphic +
Not analysed -

Table 71: Allelic frequencies of four natural populations
of P. indicus

| Gene | Alleles | <u>Populations</u> | | | |
|----------|---------|--------------------|-----------|--------|---------|
| | | Cochin | Tuticorin | Madras | Waltair |
| Acph-3 | a | 0.5278 | 0.5 | 0.5972 | 0.6528 |
| | b | 0.4723 | 0.5 | 0.4028 | 0.3472 |
| Ald-1 | a | 0.1528 | 0.1944 | 0.1806 | 0.1111 |
| | b | 0.3889 | 0.5147 | 0.5833 | 0.7778 |
| | c | 0.4583 | 0.2639 | 0.2361 | 0.1111 |
| Ao-1 | a | 0.5833 | 0.5833 | 0.6250 | 0.6528 |
| | b | 0.4167 | 0.4167 | 0.3750 | 0.3472 |
| 6-Pgdh-2 | a | 0.4306 | 0.4306 | 0.4028 | 0.4444 |
| | b | 0.5694 | 0.5694 | 0.5972 | 0.5556 |
| Alph | a | - | - | - | 0.8333 |
| | b | - | - | - | 0.1667 |
| Mdh-1 | a | 0.3472 | 0.3750 | .3056 | .3472 |
| | b | 0.6528 | 0.6250 | .6944 | .6528 |
| Me-1 | a | 0.6667 | 0.7222 | 0.7778 | 0.8056 |
| | b | 0.3333 | 0.2778 | 0.2222 | 0.1944 |
| Odh-2 | a | 0 | 0 | 0 | 0.0833 |
| | b | 1 | 1 | 1 | 0.9167 |

Table 72: Allelic frequencies of two natural populations of Parapenaeopsis stylifera.

| Gene | Alleles | Populations | |
|----------|---------|-------------|---------|
| | | Cochin | Bombay |
| Acph-2 | a | 0.7361 | 0.3472 |
| | b | 0.2639 | 0.6528 |
| Adh-2 | a | 0.9444 | 0.9028 |
| | b | 0.556 | 0.0972 |
| Ao-2 | a | 0.9583 | 0.9722 |
| | b | 0.0417 | 0.0278 |
| Ao-3 | a | 0.9722 | 0.9722 |
| | b | 0.0278 | 0.0278 |
| Alph-2 | a | 0.9167 | 0.8889 |
| | b | 0.0833 | 0.1111 |
| Est-2 | a | 0.875 | 0.9306 |
| | b | 0.125 | 0.0694 |
| Gpdh-1 | a | 0.9583 | 0.9583 |
| | b | 0.0417 | 0.0417 |
| Mdh-1 | a | 0.9305 | 0.9611 |
| | b | 0.0694 | 0.1389 |
| Me-1 | a | 0.611 | - |
| | b | 0.3889 | - |
| Odh-2 | a | 0.9722 | 0.9861 |
| | b | 0.0278 | 0.01389 |
| 1-Pydh-1 | a | 0.9583 | 0.9861 |
| | b | 0.0417 | 0.0134 |
| To-1 | a | 0.5833 | 0.5139 |
| | b | 0.4167 | 0.4861 |
| To-2 | a | 0.9583 | 0.9583 |
| | b | 0.0417 | 0.0417 |

Table 73: Average frequency of observed (\bar{H}_o) and expected (\bar{H}_e) heterozygotes per locus with 'Z' value for Penaeus indicus.

| Gene | Population | \bar{H}_o | \bar{H}_e | Z |
|----------|------------|-------------|-------------|---------|
| Acph-3 | Cochin | 0.06 | 0.498 | 9.69022 |
| | Tuticorin | 0.06 | 0.5 | 11.1167 |
| | Madras | 0.03 | 0.48 | 15.8283 |
| | Waltair | 0.03 | 0.458 | 15.0545 |
| Ald-1 | Cochin | 0.08 | 0.615 | 11.831 |
| | Tuticorin | 0.08 | 0.6 | 11.4993 |
| | Madras | 0.08 | 0.571 | 10.858 |
| | Waltair | 0.06 | 0.37 | 7.8283 |
| Ao-1 | Cochin | 0.06 | 0.486 | 10.763 |
| | Tuticorin | 0.06 | 0.486 | 10.763 |
| | Madras | 0.03 | 0.469 | 15.4414 |
| | Waltair | 0.03 | 0.453 | 14.8786 |
| 6-Pgdh-2 | Cochin | 0.03 | 0.49 | 16.18 |
| | Tuticorin | 0.03 | 0.49 | 16.18 |
| | Madras | 0.03 | 0.48 | 15.8283 |
| | Waltair | 0.05 | 0.49 | 12.114 |
| Alph | Waltair | 0.05 | 0.278 | 6.2768 |
| Mdh-1 | Cochin | 0.03 | 0.453 | 14.8786 |
| | Tuticorin | 0.03 | 0.469 | 15.441 |
| | Madras | 0.05 | 0.424 | 10.297 |
| | Waltair | 0.03 | 0.547 | 18.2042 |
| Me-1 | Cochin | 0 | 0.444 | 0 |
| | Tuticorin | 0 | 0.401 | 0 |
| | Madras | 0 | 0.346 | 0 |
| | Waltair | 0.03 | 0.313 | 11.127 |
| Odh-2 | Cochin | 0 | 0 | 0 |
| | Tuticorin | 0 | 0 | 0 |
| | Madras | 0 | 0 | 0 |
| | Waltair | 0.06 | 0.153 | 2.349 |
| Total | Cochin | 0.0114 | 0.1357 | |
| | Tuticorin | 0.0114 | 0.1339 | |
| | Madras | 0.0096 | 0.1259 | |
| | Waltair | 0.0142 | 0.1331 | |

Table 74: Average frequency of observed \bar{H}_o and expected \bar{H}_e heterozygotes per locus with 'Z' value for Parapenaeopsis stylifera.

| Gene | Population | \bar{H}_o | \bar{H}_e | Z |
|----------|------------|-------------|-------------|---------|
| Acph-2 | Cochin | 0.08 | 0.39 | 6.858 |
| | Bombay | 0.08 | 0.45 | 8.186 |
| Adh-2 | Cochin | 0 | 0.105 | 0 |
| | Bombay | 0.03 | 0.176 | 5.1408 |
| Ao-2 | Cochin | 0.03 | 0.08 | 1.76 |
| | Bombay | 0.05 | 0.054 | 0.1102 |
| Ao-3 | Cochin | 0. | 0.054 | 0 |
| | Bombay | 0.05 | 0.054 | 0.1102 |
| Alph-2 | Cochin | 0.06 | 0.153 | 2.354 |
| | Bombay | 0.06 | 0.198 | 3.4936 |
| 1-Pydh-1 | Cochin | 0.03 | 0.054 | 0.8441 |
| | Bombay | 0.03 | 0.027 | 0.1056 |
| Est-2 | Cochin | 0.08 | 0.219 | 3.0752 |
| | Bombay | 0.03 | 0.129 | 3.4859 |
| Epdh-1 | Cochin | 0.08 | 0.08 | 0 |
| | Bombay | 0.03 | 0.08 | 1.7605 |
| Mdh-1 | Cochin | 0.03 | 0.129 | 3.4859 |
| | Bombay | 0.06 | 0.239 | 4.5316 |
| Me-1 | Cochin | 0.01 | 0.475 | 7.5 |
| Odh-2 | Cochin | 0.06 | 0 | 1.51898 |
| | Bombay | 0.03 | 0.08 | 1.7605 |
| To-1 | Cochin | 0.06 | 0.486 | 10.784 |
| | Bombay | 0.03 | 0.5 | 16.549 |
| To-2 | Cochin | 0.08 | 0.08 | 0 |
| | Bombay | 0.08 | 0.08 | 0 |
| Total | Cochin | 0.03 | 0.082 | |
| | Bombay | 0.025 | 0.0984 | |

Table 75: Summary of genetic variation Data in four geographic populations of Panaeus indicus.

| | Geographic locations ^Y | | | | |
|--|-----------------------------------|-----------------|---------------|-----------------|---------|
| | Co | Tun | Mds | Wal | Mean |
| Sample size | 36 | 36 | 36 | 36 | 36 |
| Number of loci | 23 | 23 | 23 | 24 | 23.25 |
| Proportion of Polymorphic Loci(<u>P</u>) | 0.261 | 0.261 | 0.261 | 0.333 | 0.279 |
| Average frequency of heterozygosity per locus (<u>H_o</u>) | 0.0114 ±0.02 | 0.0114 ±0.02 | 0.01 ±0.02 | 0.0142 ±0.02 | 0.01175 |
| Mean number of alleles per loci(A) | 1.304 | 1.304 | 1.304 | 1.375 | 1.322 |

^YCo - Cochin, Tun-Tuticorin, Mds-Madras, Wal-Waltair

Table 76: Summary of genetic variation: data in two populations of Parapenaeopsis stylifera.

| | Geographic locations ² | | |
|--|-----------------------------------|--------------|--------|
| | Co | Bom | Mean |
| Sample size | 36 | 36 | 36 |
| Number of loci | 23 | 22 | 22.5 |
| Proportion of loci polymorphic (<u>p</u>) | 0.304 | 0.273 | 0.2885 |
| Average frequency of heterozygosity per locus (<u>H_o</u>) | 0.03 ± 0.03 | 0.025 ± 0.03 | 0.028 |
| Mean number of alleles per loci(A) | 1.545 | 1.571 | 1.558 |

²Co - Cochin; Bom-Bombay

Table 77: Nei's genetic distance (D) and genetic identity (I) analysis in Penaeus indicus collected from four different locations.

| | Cochin-Tuticorin | | Cochin-Madras | | Cochin-Waltair | | Tuticorin-Madras | | Tuticorin-Waltair | | Madras-Waltair | |
|---------------|------------------|--------|---------------|--------|----------------|--------|------------------|--------|-------------------|---------|----------------|---------|
| | I | D | I | D | I | D | I | D | I | D | I | D |
| Acph | 1 | 0 | .9903 | 0.0097 | .972 | 0.0284 | 1 | 0 | .956 | 0.0450 | .9689 | 0.0316 |
| Ald | .93502 | 0.0671 | .8933 | 0.1128 | .7525 | 0.2844 | .9973 | 0.0027 | .94 | 0.0619 | .962 | 0.0387 |
| Ac-1 | 1 | 0 | .9967 | 0.0033 | .9915 | 0.0085 | .9969 | 0.0031 | .998 | 0.0020 | .999 | 0.0010 |
| 6-Pgdh | 1 | 0 | .9984 | 0.0016 | 1 | 0 | .9719 | 0.0285 | 1 | 0 | .996 | 0.0040 |
| Mdh | .9987 | 0.0013 | .9973 | 0.0027 | 1 | 0 | .9922 | 0.0078 | .998 | 0.0020 | .9973 | 0.0027 |
| Me | .995 | 0.0050 | .9829 | 0.0172 | .9102 | 0.0941 | .9961 | 0.0039 | 1.059 | -0.0573 | 1.068 | -0.0658 |
| Odh | 1 | 0 | 1 | 0 | .9958 | 0.0042 | 1 | 0 | .996 | 0.0440 | .996 | 0.004 |

Acph-Acidphosphatase;

Ald -Aldolase

Ac-1-Aldehydeoxidase

6-Pgdh-6-Phosphoglucconate dehydrogenase

Mdh -Malatedehydrogenase

Me -Malic enzyme

Odh -Octanoldehydrogenase.

Table 78: Nei's genetic distance (D) and genetic identity(I) analysis between Cochin and Bombay samples of Parapenaeopsis stylifera.

| Enzyme Locus | Cochin-Bombay | |
|---------------------------------|---------------|--------|
| | I | D |
| Acidphosphatase (Acph) | .74 | 0.3011 |
| Alcohol dehydrogenase (Adh) | .998 | 0.0020 |
| Aldehydeoxidase (Ao-1) | .9862 | 0.0139 |
| Aldehydeoxidase (Ao-2) | .94597 | 0.0555 |
| Alkalinephosphatase (Alph) | 0.996 | 0.0040 |
| Esterase (Est-2) | .9449 | 0.0567 |
| Glycerophosphate (Gpdh) | 1 | 0 |
| dehydrogenase | | |
| Malate dehydrogenase (Mdh) | 1 | 0 |
| Octanol dehydrogenase (Odh-2) | 1 | 0 |
| 1-Pyrroline dehydrogenase(Pydh) | 1 | 0 |
| Tetrazoliumoxidase (To-1) | 1 | 0 |
| Tetrazoliumoxidase (To-2) | 1 | 0 |

Genetic variation between prawn populations:

Genetic divergence among P. indicus and P. stylifera population was quantified by Nei's measure of genetic distance (D) and Genetic identity (I) considered as the average probability per locus of selecting two electrophoretically identical alleles, one from each of 2 different populations. This is calculated directly from gene frequency data with Nei(1972) definition. Genetic distance (D) is defined as the negative natural logarithmic transformation of I and with average number of amino acid substitutions per protein that have diverged from one another (Nei 1973).

Population samples of P. indicus collected from 4 different areas are genetically similar. Genetic identity and genetic distance estimates of Nei & Roger's are given in table 67 & 68. Likewise, populations of P. stylifera which were sampled from Cochin and Bombay are also genetically identical. It shows genetic identity of 0.991 and genetic distance 0.009. According to Rogers analysis, the distance is 0.03 and similarly 'S' is 0.97. In P. indicus, the genetic similarity and distance for different enzymes of geographic populations like Cochin-Tuticorin, Cochin-Madras, Cochin-Waltair, Tuticorin-Waltair, Madras-Waltair, Tuticorin-Madras were tabulated (Table No. 77). Similarly the genetic variants calculated for different enzymes between Bombay, Cochin population of P. stylifera is given in Table 78.

Discussion:

The present observation of electrophoretic patterns of 15 different enzymes and their loci tested in eye, hepatopancreas and muscle tissues of P. indicus and P. stylifera has not been reported earlier in these two species.

Acid Phosphatase

The buffer system tris-citrate pH 7 used in the present study resolved acid phosphatase enzyme in P. indicus and P. stylifera. The search for intra species genetic variation of acid phosphatase enzyme in the prawn species P. indicus and P. stylifera expressed 5 loci in the former and 3 loci in the later in selected tissues. Electrophoretic variant forms of the enzyme was tested only in the muscle tissue of both the species. The present observation of electrophoretic variant forms of Acid phosphatase at Acph-3 and Acph-2 locus in muscle tissue of P. indicus and P. stylifera respectively has not been reported earlier. The reports of its allelic forms in eye, hepatopancreas and green glands of Homarus americanus (Tracey et al., 1975), in cephalothorax of penaeid spp. (Mulley and Latter 1980), in muscle of Penaeus kerathurus and P. japonicus (DeMatthaeis et al., 1983), in muscle tissue of crabs (Beckwitt 1985) also reveals polymorphic nature of the enzyme in many

Hedgecock et al., 1977; Berthelemy, 1978; Ayala and Valentine, 1978; Hedgecock et al., 1979; Nelson and Hedgecock, 1980) strengthens the present assumptions that the acid phosphatase variants observed in P. indicus and P. stylifera are of genetic nature. The lack of balanced polymorphism of acid phosphatase in these two species studied here may be due to the certain biological and non-biological factors such as differences in the natural selection of a particular genotype resulting in the differential mortality of such types during its life cycle, or due to ontogenetic effects. Sampling error also might contribute to this result. Breeding experiments which is a direct method for proving the genetic nature of an observed variation, is not practical and was beyond the scope of the present investigations. The lack of Acid phosphatase heterozygotes in P. indicus and P. stylifera obtained here may be also due to either inbreeding effect or higher mortality of the heterozygote individuals in the populations. Problems of inbreeding seen in the species of penaeid prawns (Mulley and Latter 1980) was due to periodic reductions of each species to a very small number of breeding individuals during glacial periods, when the ocean receded from the tidal lakes and river estuaries. Population size bottleneck involves inbreeding effect due to over-exploitation which reduces heterozygosity as in American lobster

Homarus americanus (Tracey et al., 1975). The selection of only intermoult specimens for the present investigation rules out probable role of ontogeny in the pattern of results observed. The important role of errors in the sampling, however, cannot be overlooked. A reasonable sample size for genetic analysis of electrophoretic variants may be that which represents all the possible phenotypes in its nearly natural proportions. Hedgecock et al., (1976) recommended at least 20-30 specimens to be sampled from a single location. In the present study testing of 36 numbers of P. indicus and P. stylifera specimens in each sample should have shown all the phenotypes in their natural proportions as expected. However, testing of larger sample size in the present case need not improve the result of goodness of fit test conducted in these two species because, the lack of goodness fit was clearly indicated in all the other enzymes tested. Hence the observed deficiency of heterozygotes in acid phosphatase in P. indicus and P. stylifera may be the result of inherent unknown reasons like inbreeding or even shifting of polymorphism towards monomorphism as fixing of certain loci occur due to long sustained inbreeding among the populations and thereby heterozygotes necessary for maintaining normal balanced polymorphism gradually disappear. The phenomenon of low level of heterozygotes as observed in

the present investigation has also been reported in many other decapod species (Nelson and Hedgecock 1980) especially in penaeid species of prawns (Mulley and Latter 1980). Significant, heterozygote deficiency with respect to Hardy-Weinberg expected proportion was consistently observed in Homarus americanus by Hedgecock et al., (1977) due to Wahlund effect. Trophic environment of prawn species is highly heterogenous (Moriarty 1977). Nevo (1978) found out that marine invertebrates having wide range of physical and trophic environments are characterized by low levels of heterozygosity due to selective elimination of mutational variation. Mulley and Latter (1980) observed low level of genetic variation of prawns due to low mutation rate and selective elimination of mutational variation. The observation of heterozygote deficiency and almost similar gene frequencies for the dominant alleles in population samples collected from different regions such as Cochin, Tuticorin, Madras and Waltair of P. indicus and from Cochin and Bombay of P. stylifera may suggest that all these populations represent a single breeding population. The result of tagging studies conducted by CMFRI (Vijayaraghavan et al., 1982) on P. indicus also corroborate the above conclusion.

The apparent significant differences in the distribution pattern of acid phosphatase phenotypes and its alleles between Cochin and Bombay suggests, irrespective of similarities at all other loci, that the populations of P. stylifera at Cochin and Bombay may be in the course of genetic differentiation. Nevertheless it requires analysis of larger population samples for further confirmation.

Alcohol dehydrogenase:

Alcohol dehydrogenase separated in P. indicus and P. stylifera showed good resolution in the tris boric acid Edta pH 8 buffer as reported in Jackass Morwang (Richardson 1982a) whereas this enzyme also resolved in tris maleic acid in P. latisulcatus (Richardson 1982b).

Analysis of intraspecies variation of alcohol dehydrogenase enzyme in prawn species studied here showed 3 loci in P. indicus and two loci in P. stylifera in different tissues like eye, hepatopancreas and muscle. Alcohol dehydrogenase loci observed in P. indicus was monomorphic in all the locations where as P. stylifera showed a diallelic polymorphism in Aldh-2 locus, expressed in hepatopancreas. The expression of alcohol dehydrogenase was reported in digestive glands of rock lobster (Smith et al 1980). As observed in P. indicus,

alcohol dehydrogenase had monomorphic loci in P. setiferus (Lester 1979). The species P. latisulcatus expressed alcohol dehydrogenase activity in eyes, hepatopancreas and muscle with polymorphic loci having 4 alleles in Jackass morwang (Richardson 1982a). Significant divergence from the distribution pattern expected under Hardy-Weinberg equilibrium conditions in the distribution of alcohol dehydrogenase phenotypes was observed in same species by the same authors (Jackass Morwang, Richardson 1982). P. stylifera also showed too many homozygotes. Since no significant variations in alcohol dehydrogenase gene frequencies of P. stylifera are evident, the data do not imply that the samples collected from Cochin and Bombay belonged to different subpopulations. Selection at this locus seems a more likely explanation for the observed heterozygote deficiency. Other probable causes suggested in acid phosphatase may have produced too many homozygotes in alcohol dehydrogenase in P. stylifera studied here.

Aldehyde oxidase:

Aldehyde oxidase is an extensively studied enzyme in Drosophila melanogaster (Ayala et al, 1974). Aldehyde oxidase enzyme of P. indicus and P. stylifera, studied here resolved well in the tris-glycine buffer 8.3 pH Aldehyde oxidase enzyme

of all the 13 penaeid prawn species analysed in Australian waters was resolved in tris citric acid 8.4 pH and that of P. japonicus and P. ketathurus in tris boric acid edta buffer 9.1 pH DeMatthaeis et al (1983).

Aldehyde oxidase in P. indicus tested here exhibited its activity in hepatopancreas and muscle only. Ao-3 locus was seen in muscle whereas Ao-1 and 2 were seen in hepatopancreas. Allele frequency estimate suggested a polymorphic locus Ao-1 and a monomorphic locus Ao-2 in all the localities samples for P. indicus.

In P. stylifera the aldehydeoxidase enzyme expressed 2 loci Ao-2 and Ao-3 in hepatopancreas and Ao-1 in muscle tissue. Genetic variation studies revealed 2 banded heterozygotes indicating monomeric subunit. The allelic frequency being not less than 0.95 implied the monomorphic nature of these loci. Likewise Homarus americanus had a polymorphic locus with 2 alleles (Tracy et al 1975)

Aldehydeoxidase showed monomorphic loci in many marine organisms (Redfield et al, 1980; Lester 1979, 1983; Fuller and Lester 1980; DeMatthaeis et al, 1983). In the present study also due to the excess of homozygosity the expected and observed phenotypic distribution showed significant differences in P. indicus (Ao-1) and for Cochin samples of

P. stylifera (Ao-2). The reasons for the observation of excess homozygotes in both the species studied here may be the same as suggested elsewhere.

Aldolase:

Aldolase catalyses a reaction in glycolysis and energy production. P. indicus tested here showed distinct bands in tris boric edta buffer pH 8. The enzyme was also reported to have resolved in tris citrate pH 7 in other penaeid prawns (Mulley and Latter 1980). Muscle tissues of P. indicus showed a monomer subunit with triallelic pattern revealing the polymorphic nature of Aldolase enzyme. But the reports of electrophoretic studies of the enzyme carried out in other crustaceans such as chthamalus montagu (Dando et al.,1979) Matuta planipes and Uca speciosa (Nelson and Hedgecock 1980) and Uca spinocarpa (Salmon et al.,1979) showed only monomorphic loci.

In other penaeid prawns aldolase was observed in muscle tissue (Mulley and Latter 1980). Red field et al., (1980) reported one monomorphic loci, in P. aztecus Ives and P. merquiensis. DeMatthaeis et al., (1983) showed one polymorphic locus with 2 alleles in cephalothorax tissue of P. japonicus and a monomorphic loci in P. kerathurus.

Lester (1983) viewed P. aztecus having monomorphic loci and Redfield et al., (1980) showed one locus monomorphic in P. merguensis. In their reports all the other enzymes except aldolase studied showed diallelic pattern.

Nelson and Hedgecock (1980) and Hedgecock et al., (1982) showed that glucose metabolising enzymes, Group I enzymes have correlation with environmental factors affecting their rate of polymorphism as seen in 51 species of decapods. Thus Group I enzymes has showed more alleles and more polymorphic than Group II enzymes.

In aldolase also due to the heterozygote deficiency significant difference with the expected value was observed. The same reasons mentioned in the acid phosphate may be the causative agents for this deviation. The aldolase being group I enzyme its influence on the observed deficiency of heterozygotes is also to be considered.

Alkaline phosphatase:

Alkaline phosphatase resolved well in tris citrate pH 7 buffer in P. indicus and P. stylifera as reported in other penaeid prawns (Mulley and Latter 1980) whereas in Macrobrachium obione (Trudeau 1978) and P. japonicus and P. kerathurus (DeMatthaeis et al., 1983) it has resolved in

tris maleic acid buffer. Electrophoretic variants of alkaline phosphatase were observed in muscle tissue of *P. indicus* and *P. stylifera*. Two alleles were detected in the muscle tissue of *P. indicus* and *P. stylifera* with monomeric subunit having polymorphism as reported in Australian penaeid prawns with monomer pattern showing 2 bands for heterozygote (Mulley & Latter 1980).

Due to the excess homozygotes, the goodness of fit tested for Hardy-Weinberg law showed significant variation in alkaline phosphatase enzyme. The reasons for the imbalance may be the same as explained in acid phosphatase.

Esterase:

Esterase enzyme was separated using histidine pH 7 and sodium citrate pH 7 buffers. In *P. indicus* consistent bands couldn't be observed due to lack of uniform pattern. *P. stylifera* showed 3 loci. One of the anodal zones Est-2 locus which expressed allelic variants showed a monomer structure. Esterase study reports in various other animals expressed the following features. *Daphnia magna* a monomer form with 3 alleles (Herbert and Ward 1972), in *Balanus balanoides* with 4 alleles (Flowerdew and Crisp 1975, 1976), in *Chthamalus stellatus* Est-2 locus with dimer form with 3 alleles and Est-4 locus with monomer form with 6 alleles (Juan 1976).

Esterase polymorphism was reported in prawns like Penaeus aztecus Ives; P. duorarum burkenroad and P. setiferus Linnaeus (Lester 1979) in P. kerathurus with 2 polymorphic loci and P. japonicus with one polymorphic locus (DeMatthaeis et al 1983) and M. bennettiae with one polymorphic locus having 4 alleles and P. plebejus had one polymorphic loci with 5 alleles (Mulley and Latter 1980). Esterases are often found to be weak and unstable and it may change due to nongenetic causes such as ontogenetic or physiological state (Johnson et al., 1974 Kannupandi 1980). In the present investigation esterases were found to exhibit inconsistent patterns in P. indicus. The observed esterase enzyme phenotypes produced significant variations in their expected frequency due to the excess of homozygotes.

Alpha glycerophosphate dehydrogenase:

Alpha glycerophosphate dehydrogenase plays an important role in the intermediary metabolism. Tissue expression studies carried out in P. indicus showed one region of enzyme activity in hepatopancreas. Since the allelic frequency exceeded 0.95 it was considered as monomorphic loci.

Monomorphic nature of the glycerophosphate dehydrogenase enzyme was also reported in Euphausia superba (Ayala et al., 1975)

Euphausia mucronata, E. distinguenda (Ayala and Valentine 1979) Cthamalus dalli Pilsbry and C. fissus Darwin (Hedgecock 1979). In rocklobster a single strongly staining band was seen (Smith et al., 1980) and a monomer subunit, was observed in American lobster (Odense and Anand 1978).

Penaeid prawns expressed inadequate resolution in muscle tissue (Mulley and Latter (1980) whereas P. merguensis showed one monomorphic locus for this enzyme (Redfield et al., 1980) and Macrobrachium rosenbergii also expressed one monomorphic locus in muscle.

Lactate dehydrogenase:

Lactate dehydrogenase plays an important metabolic role and exist in several isozymic forms. Zymogram patterns of this enzyme showed 2 loci in different tissues like eye, hepatopaneas and muscle in P. indicus studied here. Since these didn't exhibit any allelic variants no genetic variation studies could be carried out. In American lobster 2 loci were seen with 3 bands of enzyme activity (Odense and Anand 1978). Rock lobster had a locus in muscle tissue and in J. edwardsii this locus was weakly polymorphic but in J. novaehollandiae it is strongly polymorphic for the same 2 alleles (Smith et al., 1980)

These Lactate dehydrogenase enzyme resolved in muscle of Penaeid prawns showed 2 bands for heterozygous condition.

Lactate dehydrogenase enzymes showed monomorphic loci in surral crustaceans (Berthelemy 1978; Hedgecock et al., 1979; Fuller and Lester 1980; Nelson and Hedgecock 1980; Redfield et al., 1980).

Malate dehydrogenase:

Genetic variation studies for malate dehydrogenase were conducted on P. indicus and P. stylifera. The enzyme was resolved in tris glycine 8.3 pH buffer. Activity of this enzyme was seen in eye and muscle tissues. It gave two banded heterozygote showing a monomer structure. Mdh-1 locus in eye was found to be polymorphic in P. indicus and P. stylifera. Malate dehydrogenase enzyme separated in different animals showed one polymorphic locus in Pandalus jordani (Berthelemy 1978), Homarus americanus (Tracey et al. 1975) and surral penaeid prawns (Lester 1979 and 1983).

Penaeid prawn (Mulley and Latter 1980) and P. japonicus and P. kerathurus (DeMatthaeis et al. 1983) Pandalid shrimp (Johnson et al., 1974) expressed malate dehydrogenase activity in muscles, P. latisulcatus resolved in eyes, hepatopancreas and muscle (Richardson 1982 b).

Phenotypic distribution of malate dehydrogenase enzyme in P. indicus and P. stylifera was tested for goodness of fit and found to show significant variation. Probably due to the causes explained in acid phosphatase enzyme.

Malic enzyme:

Electrophoretic separation of this enzyme for genetic variation studies have been done on many animals. In the present study of P. indicus and P. stylifera this enzyme resolved in buffer tris maleic acid edta buffer 7.6 pH. In P. indicus 2 loci were observed one in eye and another in muscle. Me-1 locus showed polymorphism for 2 alleles. Heterozygote with 2 banded nature proved it to be having monomer structure. Eye and muscle of P. stylifera also expressed one locus. Me-1 in eye tissue tested in Cochin expressed polymorphism.

Malic enzyme resolved using tris citric acid 8.4 pH in Penaeud prawns (Mulley and Latter 1980) P. kerathurus and P. japonicus (DeMatthaeis et al., 1983) found to have one monomorphic loci in muscle tissue. Expression in rock lobster was as a diffused band (Smith et al., 1980) whereas in Pandalid shrimp (Johnson et al., 1974) showed it as an anodal band in muscle. Penaeus merguensis de Man (Hedgecock et al., 1979) Pandalus danae, P. jordani and P. platyceros (Berthelemy 1978) showed one monomorphic loci. Homarus americanus (Tracey et al., 1975) had one polymorphic loci

with 2 allele and H. gammerus (Hedgecock et al., 1977) showed a polymorphic loci with 4 alleles. Expected values of Hardy-Weinberg equilibrium expressed significant variation due to the reasons already mentioned for acid phosphatase enzyme.

Octanol dehydrogenase:

Studies on genetic variation in P. indicus and P. stylifera showed polymorphic locus in P. indicus eye tissue. This enzyme was found out to be showing a dimer pattern with three banded phenotype for heterozygote. Electrophoretic separation was done using tris maleic buffer 7.6 pH. Significant variation in the goodness of fit of phenotype distribution may be due to reasons discussed for acid phosphatase enzyme.

The interesting observation of apparent polymorphism at Octanol dehydrogenase only in the Waltair populations of P. indicus suggests that they may be an isolated population from that of Cochin, Tuticorin and Madras. Further detailed studies alone can confirm the present findings.

Reports of electrophoretic separation of this enzyme carried out in various other prawns like P. esculentus, P. latisulcatus, P. longistylus, M. macrleayi, M. insolitus, M. eboracensis and P. semisulcatus showed one polymorphic

locus with 2 alleles (Mulley and Latter (1980). P. esculentus and M. endeavouri showed 3 alleles in a polymorphic loci and P. merquiensis, M. bennettiae (Mulley and Latter 1980) and P. latisulcatus (Richardson 1982b) expressed a polymorphic locus with four alleles.

6-Phosphogluconate dehydrogenase:

The hepatopancrease in P. indicus showed 6 Phosphogluconate dehydrogenase polymorphism. Gene frequency data for this enzyme was carried out on P. indicus, collected from 4 different localities. It expressed two loci in various tissues like muscle and hepatopancreas of P. indicus. The separation of this enzyme carried out in tris boric acid edta pH 8. Activity of this enzyme was reported in various animal tissues like muscle (Redfield et al., 1980; Mulley and Latter 1980), in digestive gland and muscle of rock Lobster (Smith et al 1980) and in eyes of P. latisulcatus (Reichards 1982 b).

In Decapoda P. aztecus, P. duorarum Burkenroad and P. setiferus Linnaeus showed polymorphic loci with 3 alleles each. P. merquiensis deman (Redfield et al., 1980), M. rosenbergii deman (Hedgecock et al., 1979) Homarus americanus (Tracey et al., 1975) and H. gammarus (Hedgecock et al., 1977) showed one monomorphic locus.

Expected phenotypic distribution showed significant differences due to the reasons already discussed for acid Phosphatase enzyme.

1-Pyrroline dehydrogenase:

Genetic variation of Pyrroline dehydrogenase was carried out in P. indicus and P. stylifera species. This enzyme gave good resolution in tris boric edta 8 pH. Hepatopancreas and Muscle showed regions of this enzyme activity. Genetic analysis proved them to be monomorphic. Redfield et al., (1980) observed this enzyme, resolving in tris-citric and boric lithiumhydroxide, tris boric acid edta and tris hydrochloric acid buffers in muscle tissue, Mulley and Latter (1980) found this locus to be polymorphic in penaeus species of prawns and monomorphic in metapenaeus species of prawns and viewed the separation using tris citrate discontinuous buffer 8.4 pH.

Tetrazolium oxidase:

This enzyme was used for genetic variation studies in P. indicus and P. stylifera. It resolved in tris boric edta pH 8 buffer and showed a polymorphic enzyme in P. stylifera in hepatopancreas tissue but found out to be monomorphic in P. indicus. Two banded heterozygote pattern observed here was also reported in Penaeid prawns of

Australia (Mulley and Latter 1980). The phenotype distribution of To at locus 2 was found to be in Hardy-Weinberg equilibrium. Polymorphic loci were reported in Chthamalus stellatus (Dando et al., 1979) Euphauria superba (Ayala et al., 1975), Cambarus bartonii (Nemeth and Tracey 1979) Panulirus interruptus, and Upagebea pugettensis, whereas monomorphic loci were seen in Pandalus dane, P. jordani and P. platyceros (Berthelemy 1978).

Sorbitol dehydrogenase:

Sorbitol dehydrogenase was analysed in P. indicus using tris glycine buffer. Genetic analysis carried out in muscle tissue showed it to be monomorphic. Redfield and Salini (1980) resolved this enzyme in tris boric acid edta pH 9 in muscle tissues of Penaeid spp of prawns. Mulley and Latter (1980) found this enzyme to be polymorphic with dimer structure in muscle tissues.

Like here slight, but significant, heterozygote deficiency with respect to Hardy-Weinberg expected proportion was consistently observed in Homarus americanus (Hedgecock et al., 1977) due to Wahlund effect. The present observation of electrophoretic variant forms of all the enzymes tested in eye, hepatopancreas and muscle tissues of P. indicus and P. stylifera has not been reported earlier.

The present first report of 15 different enzyme systems and their loci in different tissues of P. indicus and P. stylifera suggests that the same enzyme can be separated and resolved suitably in different buffer systems irrespective of the species.

The individual enzyme wise discussion shows clearly that the present observations of monomorphic and polymorphic nature of enzymes in P. indicus and P. stylifera in their different tissues and distribution pattern of different phenotypes in their populations are comparable with that of other prawn species and crustaceans as reported by others. The deficiency of heterozygotes in all the enzymes except in one may be caused by one or more unknown reasons discussed.

The interesting observation of apparent polymorphism at Octanol dehydrogenase only in the Waltair populations of P. indicus suggest that they may be an isolated population from that of Cochin, Tuticorin and Madras. Further detailed studies alone can confirm the present findings.

The results of the present investigation of intra species variation of enzymes such as acid phosphatase, alcohol dehydrogenase, aldehydeoxidase, aldolase, alkaline phosphatase, esterase, α -glycerophosphate dehydrogenase,

lactate dehydrogenase, malate dehydrogenase, malic enzyme, octanol dehydrogenase, 6 phosphogluconate dehydrogenase, pyrroline dehydrogenase, sorbitol dehydrogenase, and tetrazolium oxidase in different tissues like eyes, hepatopancreas and muscles and their distribution pattern in different populations of P. indicus and P. stylifera thus become an important contribution in understanding the hitherto unknown population genetics of P. indicus and P. stylifera which are commercially important cultivable prawn species of India.

Electrophoresis gives the basic information upto genotype or allele frequencies at each locus in a given population. Different measures have been utilized to express the amount of genetic variation within a population. The most informative way of expressing this is the occurrence of heterozygosity. The proportion of polymorphic loci in a population is another measure of genetic variation commonly used. A third measure of genetic variation is the average number of alleles per locus.

Genetic differentiation among the populations can be found out using measures of Genetic similarity (I) and genetic distance (D) developed by Nei (1972, 1973). Genetic similarity is the average probability of selecting two electrophoretically identical alleles per locus from 2

different populations. Genetic distance is measured as the mean of electrophoretically detectable amino acid substitutions that have occurred since the 2 populations being compared have diverged from a common ancestor.

Mean number of alleles in a population:

Hydrobiological conditions especially temperature is found to have correlation with differential activity of electrophoretic alleles (Koehn 1969, Smith et al., 1978). This measure is highly dependent on the number of individuals studied since rare alleles are detected in larger samples and also with more number of polymorphic loci. In the present analysis of 23 loci only seven loci were found out to be polymorphic in P. indicus and six polymorphic loci were observed out of twenty two loci analysed in P. stylifera. Since majority of the loci studied in P. stylifera and P. indicus are monomorphic the average number of alleles per locus is low. In P. indicus it was found out to be 1.304 in Cochin and 1.375 in Tuticorin, Madras and Waltair. Overall mean number of alleles per locus in P. indicus was found out to be 1.322. P. stylifera showed mean number of alleles 1.545 in Cochin and 1.571 in Bombay and overall mean number of alleles per locus was 1.558. Similar low values were in H. americanus, (Tracey et al., 1975) where the average number of alleles per population is varied from 1.19 to 1.55, whereas Hedgecock et al. (1977) reported

average number of alleles to be 1.2 per locus in H. gammerus. In P. kerathurus and P. japonicus mean number of allele per locus (A) is 1.265 and 1.484 respectively (DeMatthaeis et al., 1983). In Decapoda (Penaeidae and caridea) number of alleles per locus was 1.64 (Hedgecock et al., 1982). In P. indicus and P. stylifera the reason for the observation of lower value of alleles per locus may be due to bottle neck size, that is population size which will curtail the incidence of rare allele in the animal. Average number of alleles per locus increases faster than the heterozygosity when the population is restored (Nei 1975).

In fishes comparisons of allele frequencies suggested significant differentiation among neighbouring populations (Burton and Feldman 1982). In lobsters Tracey et al. (1975) showed Mdh-2¹⁰⁵ allele at a frequency of 0.2 at Wood hole whereas the same analysed in Martha's Vineyard samples lacked that allele. The same allele has a frequency of 0.11 at the offshore LSA site but was absent from CSB site located 60 km away. Available genetic data indicate substantial differentiation among local H. americanus population despite this species' planktonic larval stages. Present study in P. indicus showed Octanol dehydrogenase enzyme, to be polymorphic in Waltair but the same was monomorphic in other locations. Though this may suggest a clear cut differentiation of

Waltair population from that of other areas, a detailed comparative similar investigation is essential for further confirmation.

Polymorphic loci:

Genetic variation measured with proportion of polymorphic loci is highly dependent on the number of individuals studied since the rare alleles are only identified when more and more samples are studied. Average proportion of polymorphic loci per population (P_p) was found out by finding average of proportion of polymorphic loci over all populations. Average proportion of polymorphic loci per population was found out to be 0.279 for P. indicus and 0.289 for P. stylifera. Proportions of polymorphic loci in P. indicus is .261 in Cochin, Tuticorin and Madras and .333 in Waltair. Thus Waltair samples showed more proportion of polymorphic loci. In P. stylifera proportion of polymorphic loci is .304 in Cochin and .273 in Bombay. Meagre variation is noticed in the two populations of P. stylifera. Mulley and Latter (1980) showed 0.14 average number of loci as polymorphic in penaeid species of prawns. Whereas in P. aztecus duorarum and setiferus 0.3 loci were polymorphic (Lester 1979). Hedgecock (1979) noticed average proportion of polymorphic gene enzyme population is 0.14

in M. rosenbergii. In H. americanus proportion of polymorphic loci ranged from 0.17 to 0.4 and from 0.154 to 0.205 in H. gammarus (Tracey et al., 1975). Penaeus kerathurus and P. japonicus showed 0.265 and 0.387 frequency of polymorphic loci (DeMattheis et al., 1983). Lester (1979) reported the proportion of polymorphic loci to be 0.33 in P. aztecus, 0.33 in P. duorarum and 0.29 in P. setiferus. Thus the low amount of polymorphism exhibited by these crustaceans investigated from others supports the present finding of similar low values in P. indicus and P. stylifera.

Hardy-weinberg law:

It is noticed that the observed distribution of phenotypes significantly deviated from the expected Hardy-Weinberg equilibrium. Population which are present nearer will have more similarity. More population of this sort deviated from Hardy-Weinberg equilibrium due to deficiency of heterozygotes and also due to different selection coefficient on particular genotype (Korpelainen 1984). Violent selectively determined oscillations on genetic frequencies marks characteristic deviations of genotypic frequencies at polymorphic loci from Hardy-Weinberg proportions (Herbert 1974). In few cases deviation from Hardy-Weinberg equilibrium is due to heterozygote deficiency

caused by temporal environmental inconsistency tend to lead a relatively lower levels of genetic diversity (Bataglia et al 1978). Significant heterozygote deficiency with respect to Hardy-Weinberg expected proportions is observed in Homarus americanus (Hedgecock et al.,1977).

Significant deviation with reference to Hardy-Weinberg law is noticed in alkaline phosphatase enzyme in Astacus leptodactylis (Romanov et al.,1976), in Esterase in Chthamalus stellatus, C. depressus (Juan 1976) in Mysis relicta (Furst and Nyman 1969) in Phosphoglucumutase enzyme in Pandalus hypsinotus (Johnson et al.,1974) and in protein of Astacus leptodactylus (Brodskii et al.,1976). In eelspout Zoarces viviparus Christiansen et al., (1977) found a significant deficit of heterozygotes for an esterase polymorphism. Ferguson (1980) suggested deviation from Hardy-Weinberg expectation in fishes is due to deficiency of heterozygote.

Heterozygosity:

The genetic variability distributed within and between populations is generally similar in P. indicus and P. stylifera. The amount of heterozygosity estimated in each population based on observed frequency of heterozygote (H_o) and expected frequency of heterozygote (H_e) was found out for P. indicus and P. stylifera (Table 73, 74). Average frequency of heterozygote per locus was also found out for each places.

In *Penaeus indicus* average frequency of heterozygote found per locus is $.0114 \pm .02$ in Cochin, 0.0114 ± 0.02 , in Tuticorin, 0.01 ± 0.02 in Madras and 0.0142 ± 0.02 in Waltair. Overall value for *P. indicus* is 0.011 ± 0.02 . In *P. stylifera* average frequency of heterozygote is $.03 \pm 0.03$ in Cochin, 0.025 ± 0.03 in Bombay and overall for *P. stylifera* it is 0.026 ± 0.03 . Observed and expected frequency of heterozygotes significantly deviates from expected for majority of the loci in *P. indicus* except for malic enzyme. In *P. stylifera* also deviation was observed in aldehydeoxidase enzyme, Pyrroline dehydrogenase, Alpha glycerophosphate dehydrogenase, Octanol dehydrogenase and tetrazolium oxidase but not in alcohol dehydrogenase in Cochin. This deviation was due to excess of homozygosity being observed in these samples. Deficiency of heterozygote was observed in Decapod crustaceans (Hedgecock et al., 1982).

Homozygosity evolves in environments with temporally seasonally fluctuating trophic resources (Valentine 1976). When the feeding habit was taken, prawns have omnivorous habits. Gopalakrishnan (1952) and Panikkar (1952) stated that food of young penaeids consist of organic detritus found on the mud algal material and other small organisms in the mud. Vegetable matters included diatoms like *coscinodiscus*, *Pleurosigma*, *Rhizosolenia*, the pelagic alga

Trichodesmium and cutting of sea weeds. The crustacean included copepods, ostracods, amphipods, tiny decapods and their larval stages, Molluscan shell pieces, polychaetes, echinoderm larvae hydroids, trematodes and foraminifera were occasionally consumed. Panikkar and Menon (1956) stated that P. indicus food consists of detritus, both animal and plant that accumulate at the bottom of their habitats which are usually areas with muddy bottom. When algal matter is available they consume it in large quantity.

Studies on phytoplankton availability in Menon 1945 proved peak season during May in Trivandrum coast. Gonzalves (1947) observed peak season during January-February in Bombay George (1953) found out a peak season in abundance during southwest monsoon. In Madras peak season is during April and May (Menon 1931), Prasad (1956) worked on the phytoplankton availability in east coast of India. Subramanyan (1959) pointed out the seasonal variation in abundance of phytoplankton. Availability of Zooplankton was seen to be showing peak season in Madras during November to February (Menon 1931) in Trivandrum the peak season is during December to February (Menon 1945). A bimodal cycle was proposed by Prasad (1954) on the availability of zooplankton during February to April and October. Prasad (1956) found a bimodal in the zooplankton distribution in Munnar and Palk Bay.

5 years study on zooplankton availability conducted by Menon and George (1977) showed the zooplankton peak during July-September and a Secondary peak in November along the south-west coast of India. All these studies proves that a variety of phytoplankton and zooplankton is available for consumption. But the availability is restricted to particular season only. Phytoplankton bloom is there during southwest monsoon in West coast and North east monsoon in East coast of India. Thus a bimodal oscillation of phytoplankton is noticed in Indian waters (Chenabhotla 1981). After this monsoon the Zooplankton increases in its quantity. So the availability of phytoplankton and zooplankton fluctuates according to season. Thus prawn is restricted with one diet for that particular season. Moriarty (1977) explains the trophic environment of prawn species to be highly heterogeneous. In this type of seasonally fluctuating trophic resource environment homozygosity evolves to a great extent (Valentine 1976). Again homozygosity excess observed in enzyme locus suggests species in inbreeding condition in natural environment (Nakajima and Masuda 1985).

Usually in large samples from random population observed and expected frequency of heterozygotes are Difference exist in observed frequency of heterozygote is due to natural selection or other factors (Ayala and Valentine 1977). Tracey et al (1975) noticed consistent deficiency of

observed heterozygote. Every population sample appears deficient of heterozygotes and thus in total H. americanus showed a slight but significant excess of homozygotes. In the 50 decapod species average heterozygosity has a mean value of nearly 5.5%. It showed low heterozygosity of 0.05-0.06 (Nelson and Hedgecock 1980) in H. americanus average number of heterozygotes per locus is 0.045. As a whole decapods are characterized by low levels of genetic variation (Hedgecock et al. 1976, Gooch 1977, Cole and Morgan 1978). Average on frequency of heterozygote seen in different prawn species is given below in the table.

Details of average frequency of heterozygotes individual per population expected at Hardy-Weinberg equilibrium (\bar{H}_e) and proportion of polymorphic loci (P).

| Species | \bar{H}_e | p |
|-------------------------------------|-------------|------|
| <u>P. setiferus</u> ₁ | 0.089 | - |
| <u>P. aztecus</u> ₁ | 0.076 | - |
| <u>P. duorarum</u> ₁ | 0.092 | - |
| <u>P. kerathurus</u> ₂ | 0.055 | 0.26 |
| <u>P. iaponicus</u> ₂ | 0.121 | 0.39 |
| <u>P. merquiensis</u> ₃ | 0.008 | 0.16 |
| <u>P. semisulcatus</u> ₃ | 0.017 | 0.22 |
| <u>P. monodon</u> ₃ | 0.008 | 0.09 |
| <u>P. esculentus</u> ₃ | 0.033 | 0.24 |
| <u>P. plebejus</u> ₃ | 0.022 | 0.24 |
| <u>P. latisulcatus</u> ₃ | 0.032 | 0.13 |
| <u>P. longistylus</u> ₃ | 0.006 | 0.08 |
| <u>M. macleayi</u> ₃ | 0.026 | 0.17 |
| <u>M. bennettiae</u> ₃ | 0.020 | 0.20 |
| <u>M. endeavouri</u> ₃ | 0.030 | 0.20 |
| <u>M. ensis</u> ₃ | 0.013 | 0.20 |
| <u>M. insolitus</u> ₃ | 0.010 | 0.10 |
| <u>M. eboracensis</u> ₃ | 0.019 | 0.17 |
| <u>P. indicus</u> ₄ | 0.011 | .28 |
| <u>P. stylifera</u> ₄ | 0.026 | .29 |

1. Lester (1979)
2. DeMatthaeis et al., 1983.
3. Mulley and Latter, 1980.
4. Present study.

Even though samples of P. indicus and P. stylifera the present studies were collected from extensive geographic area it showed low heterozygosity as seen in P. monodon and P. latisulcatus found out by Mulley and Latter (1980). Higher amount of heterozygosity is seen in P. japonicus was considered to be due to large effective population and the admixture of different geographical population (DeMatt- haeis et al, 1983). Successful and widespread marine invertebrates occupy wide range of physical and trophic environment and characterized by extremely low levels of heterozygosity (Mulley and Latter 1980) since mutational variants are selectively eliminated as seen in Australian prawn (Mulley and Latter 1980). Thus all the above said factors are main causative agents which reduces the amount of heterozygosity in P. indicus and P. stylifera population.

A significant excess of homozygote over Hardy-Weinberg expectations is commonly encountered in electrophoretic phenotypes of marine invertebrates. This might be due to preferential selection of homozygotes over the less fit heterozygotes occupying different niches. Another factor which gives rise to apparent homozygote excess is Wahlund effect. This is due to mixing of populations containing same pair of alleles at different frequencies (Crisp 1977). Hedgecock et al, (1982) observed that proportional difference between observed and expected heterozygosity is seen over all

two allele cases of polymorphism in each of 38 species of decapods. The mean differences over all species was $D = -0.009 \pm 0.010$ not significantly different from zero. So at this level no evidence for widespread subdivision of decapod population (Hedgecock et al., 1982). Mixing of prawn populations has been proved by tagging studies. New light on the migration of the P. indicus using Tagging studies revealed that Tirunelveli coast is replenished by prawns migrated from the backwaters of Cochin (Vijayaraghavan et al., 1982). Suggesting that lack of heterozygosity in P. indicus and P. stylifera as observed in the present study need not be due to subdivision and separation of these species with genetically different populations.

Enzyme function and structure have influence on mean heterozygosity (Selander, 1976). Three different classifications of enzymes into two groups have been proposed on the above basis. Glucose and non-glucose metabolising enzymes (Gillespie and Kojima, 1968). Enzymes involved in processing substrates derived from external environment and those enzymes handling substrates from internal house keeping transactions. (Johnson et al., 1973; Ayala et al., 1972). Enzymes having single and multiple specifying substrates. (Gillespie and Langley 1974). Under each of the above classification mean heterozygosity

is greater in the second group of enzymes rather than first group of enzymes (Selander 1976). Vertebrates didn't show any significant differences between glucose and non-glucose metabolising enzyme heterozygosities, but invertebrates show differences.

Mean heterozygosities in glucose-metabolising (Group I) and non-glucose metabolising enzymes (Group II) found in *P. indicus* and *P. styliifera* is given below:

| Protein | Heterozygosity | |
|---|--------------------------|---|
| | <u><i>P. indicus</i></u> | <u><i>P. styliifera</i></u> |
| Glucose-metabolising enzyme (Group I) | | |
| Aldolase | 0.08 | |
| Alpha Glycerophosphate dehydrogenase | | 0.06 |
| Malate dehydrogenase | 0.03 | 0.04 |
| Malic enzyme | 0.0069 | 0.1 |
| 6-Phosphogluconate dehydrogenase | 0.03 | |
| Non-glucose metabolising enzymes (Group II) | | |
| Alcohol dehydrogenase | | 0.014 |
| Acid phosphatase | 0.04 | .08 |
| Esterase | | 0.06 |
| Tetrazoliumoxidase | | To ₁ - 0.08, To ₂ - 0.04 |
| Octanol dehydrogenase | 0.014 | 0.04 |
| Pyrroline dehydrogenase | | 0.054 |
| Alkaline phosphatase | 0.05 | 0.06 |
| Aldehyde oxidase | Ao-1 0.04 | Ao-1 0.04 Ao ₂ - 0.03 |

From the above results it is revealed that P. indicus showed lesser heterozygosity in aldolase, malate dehydrogenase, malic enzyme and 6-phosphogluconate dehydrogenase which are glucose metabolising enzymes and more heterozygosity in acid phosphatase, octanol dehydrogenase, alkaline phosphatase and aldehyde oxidase which are non-glucose metabolising enzymes. P. stylifera shows more heterozygosity in many of the non-glucose metabolising enzymes like alcohol dehydrogenase, acid phosphatase, esterase, tetrazolium oxidase, octanol dehydrogenase, 1-Pyrroline dehydrogenase, alkaline phosphatase and aldehyde oxidase and little heterozygosity in some of the glucose metabolising enzymes like glycerophosphate dehydrogenase, malate dehydrogenase and malic enzyme. Thus the above results reveals the difference between glucose and non-glucose metabolising enzyme heterozygosities. Thus shows the more variable enzyme in Group II enzymes and less variable enzymes in Group I enzyme.

Nei's identity is usually less than 0.75 between species. Between population of a single species the level of genetic identity is usually 0.90 or greater (Nei, 1973)

Ayala (1975) found out Limulus polyphemus, Phoronopsis viridis and Tridacna mixima to be having identity 0.99, 0.96 and 0.968 and deviation to be 0.01, 0.004 and 0.032 for their populations. The values obtained in the present

study (Tables 67 & 68) reveal that they belong to one and the same species. Population collected have more or less similar allele frequencies for polymorphic loci.

Genetic identity and deviation analysis reveal that prawns P. indicus and P. stylifera collected from different populations belong to the one and the same respective species. Only a small proportion of loci were found to have alleles at significantly different frequencies. Populations regarded as subspecies generally show four times as much genetic divergence as geographically separated but morphologically similar one.

Population size has been postulated as influencing heterozygosity (Soule 1976). Small population size associated with specialised local or isolated population may theoretically limit heterozygosity (Kimura and Ohta, 1971; Nei, 1975). P. japonicus showed high heterozygosity due to large effective population size (DeMatthaeis et al., 1983).

In the present study 36 numbers of specimens were collected in each location for electrophoretic analysis where as Hedgecock et al., (1976) suggested atleast 20-30 individuals to be sampled from a single location. Hence, the sample size of present investigation might not have effect on the expected results.

Several models of genetic variability and heterozygosity and their predictions for environments or species with low and high levels of genetic variation has been proposed by many authors. According to the size and mobility Selander and Kaufman (1973) proposed that small and sessile organism to have more genetic variation than large and mobile organisms. By taking into consideration of trophic resources stability Ayala and Valentine (1977) explained that high seasonality to exhibit low genetic variation whereas low seasonality to show high genetic variation.

By environmental heterogeneity Levins (1968) characterised habitat specialists to show high levels and habitat generalists by low levels of genetic variation. Difference in heterozygosity between specialists and generalists was explained through environmental heterogeneity. A specialists species perceives its environment as coarse grained or heterogeneous having common, widespread, broad niched, main land species and will be affected by the environment. A generalist species perceives its environment as fine grained or homogenous (Smith and Fujio 1982). Any difference in this environment is of minor importance to the animal. Thus a fine grained environment for one species may be a course-grained environment for the other species (Valentine 1976).

Most of the prawn species occupy an extensive geographic area like P. indicus found in Indo-Pacific, S. Africa to China, New Guinea and Australia and P. stylifera seen in Indo-West Pacific. Besides this they have mixed life cycle, to include a broad-niched species. Long larval period faced by these animals result in unpredictable environment with consequent selection of few alleles.

Life cycle of P. indicus is completed after passing through two distinct environments the sea and the estuary. The larval development takes place in the sea and the migration into the estuaries, lakes and backwaters commences when they are in late mysis or early post-larval stages (Mohamed 1970)

Berger's (1973) findings in genus *Littorina* supports the inverse relationship between the capacity of larval dispersal and the extent of population. Genetic differentiation proposed by Gooch and Schopf (1972) and Snyder and Gooch (1973) compared L. saxatilis (no planktonic larvae) to Nassarius obsoletus (with long lived planktonic larvae) and found significantly greater differentiation in the former species. Hence Barton (1983) expected that adults which are sedentary and lack planktonic larvae always found to show significant differentiation of population on a relatively small geographical scale. Burton (1983) found limited geneflow among geographically separated invertebrates.

Smith et al, (1980) showed that long larval phase is a potential for extensive gene flow, suggesting that stock differences are unlikely. Turner and Lyster (1980) proved that exchange of pelagic larvae is found to be the reason for the genetic similarity whereas New Zealand Shapper which has a short larval stage is divided into a number of discrete stocks (Smith 1979). Tracey et al (1975) showed large, mobile, generalised lobsters adapted to temporarily and spatially varying environment through phenotypic variability rather than genetic variability. As the larval stages of the two species P. indicus and P. stylifera studied here are of greatly mobile in nature lack of significant genetic variability presently observed shows their different populations comparable to that of lobsters.

Mixed life cycle in decapods shows a change during the life cycle from a pelagic and planktonic larva to a free swimming adult. This life cycle heterogeneity might force the animal to perceive its environment as coarse-grained and select a few generalised rather than many specialised alleles. Because of this a low level of genetic variation have been found in decapods with a more heterogeneous life cycle. The tropic environment of prawn is shown to be highly heterogeneous (Moriarty 1977) Marine invertebrate species such as thirteen penaeid prawn species

subject to wide range of physical and trophic environments were characterized by low levels of heterozygosity (Mulley and Latter 1980).

According to the environment the specialist species, select several narrow-range alleles and is characterized by high heterozygosities. In the generalists the individuals bear "flexible" alleles which are few and wide ranged alleles characterized by low heterozygosities (Smith and Fugio 1982). This same observation was seen in P. indicus and P. stylifera. Specialists species feed on narrow range of food, whereas generalists consume a wider range of food. In this way high heterozygosity is seen in specialists such as Aulorhynchus flavidus (Hart 1973) and low heterozygosity such as Enophrys bison feeding on algae and hard and soft invertebrates (Hart 1973). Penaeid prawns can be included in generalists which occupy wide range environment and feed and exhibit low levels of heterozygosity.

A hybrid environmental heterogeneity trophic diversity model has been proposed to explain genetic variability in decapod crustacea (Nelson and Hedgecock 1980). In 51 species of coastal, intertidal, temperate and tropical decapods, the specialist species which are characterized by small, less mobile animals occupying a number of sub niches has high variability in Group I enzymes and low variability in Group II

enzyme and thus act as trophic specialists with narrow range of substrates promoting low variability. In the same way the generalist decapods, species with a fine-grained are characterized by low genetic variation in Group I and high genetic variation, in Group II enzymes. These include large, mobile crustaceans like prawns. As trophic generalists they face a wide variety of food species and heterogeneous tropic environment (Moriarty 1977) showing high variability in the external substrate (Group II) enzymes. This habitat specialist-generalist model would classify the decapods as generalists and observed low variability (Valentine 1976) which is observed in the case of P. indicus and P. stylifera analysed in the present study.

A relationship between heterozygosity and the level of variation of environmental factors as well as the variation in morphological traits was established (Johnson and Mickevich 1977). Several studies have shown correlations between water temperature or latitude and frequencies of electromorphs in marine animals (Johnson, 1971, 1974, 1977; Mitton and Koehn, 1975; Powers and Powers, 1975;) corresponding biochemical differences have been demonstrated between genotypes (Koehn 1969, Powers and Powers 1975). Allele frequencies at enzymes were related to hydrobiological conditions (Koehn 1969 Smith et al., 1978; Smith 1979).

Under extreme conditions such as high temperature oxygen deficiency different genotypes possessing varying viability (Kirpichnickov 1981). Relationships between biochemical variability and environment appear to be very complex in nature. This is supported by evidence shown that variation in Lactate dehydrogenase and haemoglobin may depend on fluctuations in many environmental factors - water temperature, oxygen content, pH, salinity and others (Powers, 1980). For polymorphism constantly fluctuating conditions of intracellular metabolism may also play an important part (Johnson 1976).

The temperature conditions of life for most species of animals, plants and micro-organism correlate with the heat stability of proteins (Alexandrove 1975); speciation appear to be accompanied by heredity changes in the heat stability of protein molecules. Snith et al., 1980; showed that in an open loop system gene flow tend to erode the different selective pressure which exerts on the animal and the selection will be for few generalised alleles rather than many specific alleles observed herein P. indicus and P. stylifera which showed 2 alleles in majority of the enzymes except Aldolase in P. indicus.

The following important conclusions are derived from the intra species genetic variation studies conducted on the prawn species P. indicus and P. stylifera.

Electrophoretic investigations of different isozymes in these two species, have enabled to detect seven polymorphic loci in P. indicus and six polymorphic loci in P. stylifera out of 23 and 22 loci analysed in the respective species, resulting in low average number of alleles per locus in both species. The lack of goodness fit as per Hardy-Weinberg equilibrium, in the distribution of different phenotypes in all the population of P. indicus and P. stylifera tested in the present study was due to deficiency of heterozygotes and excess of homozygotes.

One or more unknown factors or models such as small sample size, preferential selection of phenotypes, temporal and seasonal fluctuations of feed, nature of function and structure of enzymes tested, environmental heterogeneity etc. might have produced the observed significant deficiency of heterozygotes and excess of homozygotes in P. indicus and P. stylifera tested in the present investigation. The values of gene frequencies, average number of alleles per locus, average proportion of polymorphic loci per populations, average frequency of heterozygotes per locus and the values of genetic distance of populations being non-significant in different populations of P. indicus and P. stylifera collected from different regions indicate that the populations

tested are not genetically different. The present findings thus support the tagging results that P. indicus populations Tirunelveli coast is actually replenished by migrated prawns from Cochin.

Thus the present study shows little evidence that the prawns P. indicus and P. stylifera are subdivided into two or more genetic stocks. For management purpose all the population of Cochin of these species of prawns can be treated as one biological unit.

MORPHOMETRY IN RELATION TO GENETIC VARIATION

Resume of literature:

Organisms occur more or less in distinct or discrete population or stocks. Stock has to be defined as a group or population of a species maintaining one or more common characteristics depending on the type of environment of domicile. (Kutkuhn 1981). Two or more unit stocks can be satisfactorily demonstrated by applying any of the following techniques such as biochemical, immunological, serological, behavioural, morphometric, meristic, mark recapture, electrophoretic etc.

Morphological variation in Zoarces viviparus was observed by Schmidt (1917a, 1918) from 61 different locations. The characters are heritable and are reported to have direct environmental influence (Schmidt 1917a, 1917b; 1918, 1920 1921a; 1921b). Morphological variation was analysed in three races of kokanee Oncorhynchus nerka (Vernon 1957), in mountain white fish Prosopium williamsoni (Holt 1960), in three sympatric Arctic cod fishes of the genera Arctogadus and Gadus (Boulva 1972) in Upeneus sulphureus (Cuvier) from Maharashtra coast (Musharraf Ali 1978) in Sardinella sirmwal from Andaman Sea (Abidi et al., 1978-79), in Lactarius lactarius

(Choudhary and Dwivedi 1980-81), and in pink salmon Oncorhynchus gorbuscha (Beacham 1985). Morphometric technique has been used for stock separation in several species of fishes including Salmo salar (Lear and Misra 1978; Riddell and Leggett 1981), Mallotus villosus (Sharp et al., 1978), Salvelinus malma (Morrow 1980) and Coregonus spp. (Casselman et al., 1981, Ihssen et al., 1981; Todd et al., 1981). The main advantage of this method is that it effectively isolate shape differences than most of the other traditional methods (Ihssen et al., 1981).

In crustaceans also some work has been done using morphological characters for stock delineation. Metric variation in populations of Carcinus maenas (William and Needham 1941) and geographic morphometric variation in American lobster Homarus americanus (Templeton 1935; Saila and Flowers 1969) were noticed.

Geographical variation using morphological characters was seen in the genus Nematocelis (Crustacea: Euphausiidae) by Gopalakrishnan (1974), in isopod Sphaeroma rugicauda by Heath (1975), in Western Atlantic population of Gammarus oceanicus segerstrale (Amphipoda) by Croker and Gable (1977), in the dwarf cray fish by Chambers et al., (1979), in Sphaeroma serratum (Isopoda) by Consiglio and Argano (1968)

and in Pontinella dara (Copepoda) by Fleminger and Hulsemann (1974).

Morphological variance was used in prawns to find intra species variation in Macrobrachium rosenbergii de man by Lindenfelser (1980), to analyse specific variation in fresh water prawn M. niloticum in lake chad and Lake Rudolf by Williamson (1972), in Penaeus semisulcatus by Morgan (1982) and P. vannamei and P. stylirostris by Lester (1983). Lui (1979) after measuring and statistically testing the morphological variables in Macrobrachium australiense disapproved Rick's (1951) suggestion of possible subdivisions.

Morphological relationship of Penaeus semisulcatus Metapenaeus affinis and Parapenaeopsis stylifera was found out by Farmer (1986). Besides morphometry in some organisms electrophoresis also gained momentum to identify different stocks as seen in Cynoglossus bilineatus from Bombay waters (Kasinathan et al., 1972), in rainbow trout Salmo gairdneri (Gjedrem and Skjesvold 1972), in four population of Menidia (Mickeyvich and Johnson 1976) in Gasterosteus aculeatus (Bell 1976), in fiddler crab Uca (Selander et al., 1971; Salmon et al., 1979) in Chthamalus montagu (Crustacea: Cirripedia) in the Adriatic (Dando et al., 1979) and in Atlantic shore crab (Davidson et al., 1985).

Several causative agents like environmental factors play crucial role in the speciation process as seen in Malaysian prawn Macrobrachium rosenbergii (de man) reared in earthen ponds in South Carolina (Smith et al., 1978). Particularly salinity variation and diet cause differences in M. carcinus from Barbados and Jamaica (Choudhury 1971) and in species of M. rosenbergii, M. javanicum and M. pilimanum (Johnson 1960) and in laboratory population of brown shrimp Penaeus aztecus (Venkataramiah et al., 1975). Maturation and spawning was found to exhibit difference between Costa Rican and Mexican P. stylirostris (Brown et al., 1980).

Results:

Univariate and Multivariate analysis were carried out for the 3 geographical population (Cochin, Tuticorin and Madras) of P. indicus (Table No. 79, 81 & 83-85) and 2 geographical population (Cochin and Bombay) of P. styliifera (Table No. 82, 86 & 87).

Table 79: Comparison of morphometric variables of Penaeus indicus samples from Cochin and Tuticorin

| Character | Sampling Location | Sample size range | Mean value | S.D. | 't' value |
|-----------|-------------------|-------------------|------------|---------|-----------|
| SSL | Cochin | 9-15 mm | 11.2639 | 1.3065 | 3.3498* |
| | Tuticorin | 10-15 mm | 12.278 | 1.262 | |
| FSL | Cochin | 6-14 mm | 8.3889 | 1.4595 | 2.4501* |
| | Tuticorin | 6-14 mm | 9.361 | 1.881 | |
| PCL | Cochin | 19-32 mm | 22.1667 | 2.3115 | 1.4761 |
| | Tuticorin | 18-29 mm | 23.056 | 2.779 | |
| CW | Cochin | 10-18 mm | 11.4028 | 1.553 | 0.4303 |
| | Tuticorin | 8-15 mm | 11.569 | 1.72 | |
| FLF | Cochin | 3.5-7 mm | 5.0556 | 0.8348 | 1.3871 |
| | Tuticorin | 6-11 mm | 5.333 | 0.862 | |
| SSD | Cochin | 7-14 mm | 9.0139 | 1.2506 | 0.4705 |
| | Tuticorin | 6-11 mm | 9.153 | 1.258 | |
| SAD | Cochin | 10-16 mm | 11.3611 | 1.3764 | 0.1526 |
| | Tuticorin | 8-15 mm | 11.417 | 1.713 | |
| AAC | Cochin | 34-54 mm | 39.5833 | 3.9668 | 0.3451 |
| | Tuticorin | 31-49 mm | 39.944 | 4.858 | |
| PAC | Cochin | 25-41 mm | 30.0556 | 2.8977 | 1.439 |
| | Tuticorin | 20-36 mm | 28.917 | 3.76 | |
| TW | Cochin | 7-28 gm | 13.1111 | 11.2282 | 0.0168 |
| | Tuticorin | 7-19 gm | 13.078 | 3.656 | |
| TL | Cochin | 101-150mm | 115.0000 | 9.2921 | 0.6397 |
| | Tuticorin | 90-140mm | 116.667 | 12.574 | |

* Significant at 5% level.

Table 8'0: Comparison of morphometric variables of Peneus indicus samples from Cochin and Madras

| Character | Sampling Location | Sample size range | Mean Value | S.D. | 't' Value |
|-----------|-------------------|-------------------|------------|---------|-----------|
| SSL | Cochin Madras | 9-15 mm | 11.2639 | 1.3065 | 4.8557* |
| | | 10-15 mm | 12.681 | 1.166 | |
| PSL | Cochin Madras | 6-14 mm | 8.3889 | 1.4595 | 6.216* |
| | | 8-14 mm | 10.472 | 1.383 | |
| PCL | Cochin Madras | 19-32 mm | 22.1667 | 2.3115 | 4.133* |
| | | 17-29 mm | 24.458 | 2.392 | |
| CW | Cochin Madras | 10-18 mm | 11.4028 | 1.5530 | 3.6058* |
| | | 8-15 mm | 12.667 | 1.419 | |
| FLF | Cochin Madras | 3.5-7mm | 5.0556 | 0.8348 | 2.8325* |
| | | 4-7 mm | 5.611 | 0.829 | |
| SSD | Cochin Madras | 7-14 mm | 9.0139 | 1.2506 | 2.8624* |
| | | 12-18 mm | 9.889 | 1.342 | |
| SAD | Cochin Madras | 10-16mm | 11.3611 | 1.3764 | 4.4192* |
| | | 9-14.5mm | 12.778 | 1.344 | |
| AAC | Cochin Madras | 34-54 mm | 39.5833 | 3.9668 | 3.8073* |
| | | 32-52 mm | 43.0000 | 3.641 | |
| PAC | Cochin Madras | 25-41 mm | 30.0556 | 2.8977 | 1.4759 |
| | | 20-39 mm | 31.083 | 3.008 | |
| TW | Cochin Madras | 7-28 gm | 13.1111 | 11.2282 | 1.16 |
| | | 5.3-20.5gm | 15.375 | 3.326 | |
| TL | Cochin Madras | 101-150mm | 115.0000 | 9.2921 | 1.4752 |
| | | 110-142mm | 120.683 | 21.165 | |

* Significant at 5% level.

Table 81: Comparison of Morphometric variables of Penaeus indicus samples from Tuticorin and Madras.

| Character | Sampling Location | Sample size range | Mean Value | S.D. | 't' value |
|-----------|-------------------|-------------------|------------|--------|-----------|
| SSL | Tuticorin | 10-15 mm | 12.278 | 1.262 | 1.4074 |
| | | 10-15 mm | 12.681 | 1.166 | |
| FSL | Tuticorin | 6-14 mm | 9.361 | 1.881 | 2.8552* |
| | | 8-14 mm | 10.472 | 1.383 | |
| PCL | Tuticorin | 18-29 mm | 23.056 | 2.779 | 2.2942* |
| | | 17-29 mm | 24.458 | 2.392 | |
| CW | Tuticorin | 8-15 mm | 11.569 | 1.72 | 2.9546* |
| | | 8-15 mm | 12.667 | 1.419 | |
| FLF | Tuticorin | 6-11 mm | 5.333 | 0.862 | 1.3947 |
| | | 4-77 mm | 6.611 | 0.829 | |
| SSD | Tuticorin | 6-11 mm | 9.153 | 1.258 | 2.4007* |
| | | 12-18 mm | 9.889 | 1.342 | |
| SAD | Tuticorin | 8-15 mm | 11.417 | 1.713 | 3.7505* |
| | | 9-14.5mm | 12.778 | 1.344 | |
| AAC | Tuticorin | 31-49 mm | 39.944 | 4.858 | 3.0203* |
| | | 32-52 mm | 43.0000 | 3.641 | |
| PAC | Tuticorin | 20-36 mm | 28.917 | 3.760 | 2.699* |
| | | 20-39 mm | 31.083 | 3.008 | |
| TW | Tuticorin | 7-19 gm | 13.078 | 3.656 | 2.7885* |
| | | 5.3-20.5gm | 15.375 | 3.326 | |
| TL | Tuticorin | 90-140mm | 116.667 | 12.574 | 0.9788 |
| | | 110-142mm | 120.683 | 21.165 | |

* Significant at 5% level.

Table 82: Comparisons of morphometric variables of Parapenaeopsis stylifera samples from Cochin and Bombay.

| Character | Sampling location | Sample size range | Mean value | S.D. | 't' value |
|-----------|-------------------|-------------------|------------|---------|-----------|
| SSL | Cochin | 8-11 mm | 9.7778 | 0.7215 | 1.4461 |
| | Bombay | 9-11 mm | 9.5139 | 0.8236 | |
| FSL | Cochin | 4-9 mm | 6.5000 | 0.94111 | 5.8919* |
| | Bombay | 6-9 mm | 7.7222 | 0.8145 | |
| PCL | Cochin | 20-29 mm | 23.8611 | 2.8601 | 1.2023 |
| | Bombay | 18-29 mm | 23.0278 | 3.0189 | |
| CW | Cochin | 9-15 mm | 11.5556 | 1.5389 | 0.6055 |
| | Bombay | 10-16 mm | 11.7639 | 1.3757 | |
| FLF | Cochin | 4-6 mm | 5.1667 | 0.5606 | 0.5247 |
| | Bombay | 3-7 mm | 5.2500 | 0.7700 | |
| SSD | Cochin | 7-11 mm | 9.3611 | 1.0185 | 3.3655* |
| | Bombay | 7-10 mm | 8.5278 | 1.0820 | |
| SAD | Cochin | 8-12 mm | 10.3889 | 0.9344 | 1.3219 |
| | Bombay | 8-12 mm | 10.0694 | 1.1094 | |
| AAC | Cochin | 23-40 mm | 31.8611 | 3.4654 | 0.7885 |
| | Bombay | 26-37 mm | 31.2778 | 2.7735 | |
| AAC | Cochin | 22-30 mm | 26.1667 | 2.3845 | 1.9530* |
| | Bombay | 21-30 mm | 25.0833 | 2.3223 | |
| TW | Cochin | 4.1-10gm | 6.1111 | 1.6039 | 0.5892 |
| | Bombay | 4-9 gm | 6.3333 | 1.5959 | |
| TL | Cochin | 86-116mm | 97.75 | 8.1744 | 0.0129 |
| | Bombay | 82-117mm | 97.7222 | 10.0046 | |

* Significant at 5% level.

Table 83: Matrix of correlation coefficient among eleven morphological variables in Penaeus indicus collected at Cochin.

| | SSL | FSL | PCL | CW | FLF | SSD | SAD | AAC | PAC | TW | TL |
|-----|---------|--------|--------|--------|---------|--------|--------|--------|--------|--------|--------|
| SSL | 1.0000 | | | | | | | | | | |
| FSL | 0.5365 | 1.0000 | | | | | | | | | |
| PCL | 0.7608 | 0.6916 | 1.0000 | | | | | | | | |
| CW | 0.7666 | 0.7861 | 0.8981 | 1.0000 | | | | | | | |
| FLF | 0.6411 | 0.5680 | 0.6910 | 0.6324 | 1.0000 | | | | | | |
| SSD | 0.8589 | 0.7248 | 0.8665 | 0.9054 | 0.6697 | 1.0000 | | | | | |
| SAD | 0.7240 | 0.7103 | 0.7529 | 0.8456 | 0.6783 | 0.8352 | 1.0000 | | | | |
| AAC | 0.7881 | 0.6851 | 0.7946 | 0.8536 | 0.6327 | 0.8996 | 0.8185 | 1.0000 | | | |
| PAC | 0.7092 | 0.6568 | 0.7685 | 0.8552 | 0.5243 | 0.8591 | 0.7757 | 0.8521 | 1.0000 | | |
| TW | -0.0298 | 0.2222 | 0.1371 | 0.1633 | -0.0037 | 0.0380 | 0.1230 | 0.1066 | 0.1746 | 1.0000 | |
| TL | 0.7849 | 0.7163 | 0.8327 | 0.8811 | 0.6704 | 0.9220 | 0.8511 | 0.9054 | 0.8425 | 0.1235 | 1.0000 |

Table 84: Matrix of correlation coefficient among eleven morphological variables in Penaeus indicus collected at Tuticorin.

| | SSL | FSL | PCL | CW | FLF | SSD | SAD | AAC | PAC | TW | TL |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| SSL | 1.000 | | | | | | | | | | |
| FSL | 0.751 | 1.000 | | | | | | | | | |
| PCL | 0.828 | 0.776 | 1.000 | | | | | | | | |
| CW | 0.692 | 0.774 | 0.924 | 1.000 | | | | | | | |
| FLF | 0.779 | 0.726 | 0.803 | 0.745 | 1.000 | | | | | | |
| SSD | 0.755 | 0.743 | 0.864 | 0.902 | 0.676 | 1.000 | | | | | |
| SAD | 0.711 | 0.701 | 0.835 | 0.877 | 0.619 | 0.884 | 1.000 | | | | |
| AAC | 0.846 | 0.793 | 0.893 | 0.848 | 0.769 | 0.876 | 0.792 | 1.000 | | | |
| PAC | 0.806 | 0.760 | 0.881 | 0.845 | 0.820 | 0.882 | 0.746 | 0.895 | 1.000 | | |
| TW | 0.732 | 0.732 | 0.930 | 0.958 | 0.776 | 0.878 | 0.892 | 0.823 | 0.847 | 1.000 | |
| TL | 0.813 | 0.771 | 0.896 | 0.899 | 0.778 | 0.898 | 0.871 | 0.895 | 0.856 | 0.909 | 1.000 |

Table 85: Matrix of correlation coefficient among eleven morphological variables in Penaeus indicus collected at Madras.

| | SSL | FSL | PCL | CW | FLF | SSD | SAD | AAC | PAC | TW | TL |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| SSL | 1.000 | | | | | | | | | | |
| FSL | 0.645 | 1.000 | | | | | | | | | |
| PCL | 0.833 | 0.736 | 1.000 | | | | | | | | |
| CW | 0.650 | 0.679 | 0.909 | 1.000 | | | | | | | |
| FLF | 0.518 | 0.626 | 0.709 | 0.609 | 1.000 | | | | | | |
| SSD | 0.561 | 0.483 | 0.699 | 0.734 | 0.435 | 1.000 | | | | | |
| SAD | 0.450 | 0.473 | 0.686 | 0.716 | 0.446 | 0.421 | 1.000 | | | | |
| AAC | 0.872 | 0.743 | 0.879 | 0.755 | 0.573 | 0.623 | 0.593 | 1.000 | | | |
| PAC | 0.765 | 0.656 | 0.842 | 0.753 | 0.655 | 0.692 | 0.535 | 0.814 | 1.000 | | |
| TW | 0.709 | 0.676 | 0.912 | 0.943 | 0.658 | 0.779 | 0.721 | 0.755 | 0.769 | 1.000 | |
| TL | 0.495 | 0.270 | 0.425 | 0.315 | 0.186 | 0.325 | 0.293 | 0.544 | 0.378 | 0.315 | 1.000 |

Table 86: Matrix of correlation coefficient among eleven morphological variables in Parapenaeopsis stylifera collected at Cochin.

| | SSC | FSL | PCL | CW | FLF | SSD | SAD | AAC | PAC | TW | TL |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| SSL | 1.0000 | | | | | | | | | | |
| FSL | 0.1683 | 1.0000 | | | | | | | | | |
| PCL | 0.3861 | 0.5679 | 1.0000 | | | | | | | | |
| CW | 0.3459 | 0.5721 | 0.8879 | 1.0000 | | | | | | | |
| FLF | 0.4473 | 0.4332 | 0.4782 | 0.4857 | 1.0000 | | | | | | |
| SSD | 0.6177 | 0.4918 | 0.7828 | 0.7980 | 0.5421 | 1.0000 | | | | | |
| SAD | 0.6828 | 0.4224 | 0.6623 | 0.6403 | 0.6364 | 0.8990 | 1.0000 | | | | |
| AAC | 0.1587 | 0.4775 | 0.7533 | 0.7757 | 0.4535 | 0.6056 | 0.5378 | 1.0000 | | | |
| PAC | 0.2546 | 0.4711 | 0.6906 | 0.6592 | 0.5985 | 0.5392 | 0.4959 | 0.7394 | 1.0000 | | |
| TW | 0.4441 | 0.5981 | 0.7521 | 0.8193 | 0.5444 | 0.7163 | 0.7101 | 0.7791 | 0.7443 | 1.0000 | |
| TL | 0.4844 | 0.5589 | 0.8295 | 0.8472 | 0.4645 | 0.6975 | 0.6715 | 0.7300 | 0.6486 | 0.8436 | 1.0000 |

Table 87: Matrix of correlation coefficient among eleven morphological variables in Parapenaeopsis stylifera collected at Bombay.

| | SSL | FSL | PCL | CW | FLF | SSD | SAD | AAC | PAC | TW | TL |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| SSL | 1.0000 | | | | | | | | | | |
| FSL | 0.6447 | 1.0000 | | | | | | | | | |
| PCL | 0.7065 | 0.5028 | 1.0000 | | | | | | | | |
| CW | 0.6460 | 0.3732 | 0.8960 | 1.0000 | | | | | | | |
| FLF | 0.5125 | 0.4783 | 0.5009 | 0.5428 | 1.0000 | | | | | | |
| SSD | 0.6809 | 0.4953 | 0.7914 | 0.7003 | 0.5573 | 1.0000 | | | | | |
| SAD | 0.7259 | 0.5278 | 0.8184 | 0.7411 | 0.4808 | 0.8969 | 1.0000 | | | | |
| AAC | 0.6487 | 0.5790 | 0.8419 | 0.8264 | 0.5954 | 0.7876 | 0.8107 | 1.0000 | | | |
| PAC | 0.6641 | 0.5563 | 0.7251 | 0.7665 | 0.5473 | 0.6643 | 0.7795 | 0.8347 | 1.0000 | | |
| TW | 0.7256 | 0.4799 | 0.8852 | 0.8765 | 0.5743 | 0.7871 | 0.8257 | 0.8635 | 0.7933 | 1.0000 | |
| TL | 0.6628 | 0.4811 | 0.8810 | 0.8462 | 0.4952 | 0.7451 | 0.7998 | 0.8647 | 0.8360 | 0.8588 | 1.0000 |

Discussion:

Various population parameters and physiological, behavioral, morphometric, meristic, calcareous, biochemical and cytogenetic characters have been used to identify fish stocks. Population measures are useful primarily for the recognition of punitive stocks at the practical management level. Application of morphometric and meristic characters in stock identification is complicated by the fact that phenotypic variation in these characters has not been directly related to particular differences in the genome (Clayton, 1981). Effects of physiological and epigenetic constraints on morphology is directly related to certain environmental parameters such as temperature and oxygen (Martin 1949, Gould 1977, Stanley 1979 and Todd *et al.*, 1981). The number of serially repeated characters alters with the environmental changes associated with attitude (Taning 1952; McGlade 1981).

Morphological (morphometric) characters represent a series of measured variables and represent the synergism between shape and size. Using these morphological characters differentiation of stocks is likely to be subtle as seen in most fish species since it is affected by allometry (Gould 1966, Sweet 1980).

Fish stocks appear to develop as a result of complex interaction between genetic (biochemical level), organismic (level of morphology, physiology and behaviour) and ecological factors (Lindsey 1981; Clayton 1981).

Multivariate comparisons of morphological measurement among closely related group of organism reflect some biological variables such as growth rate or sexual dimorphism (e g. Eyles and Blackith 1965) According to the present study a large portion of morphometric variation among stocks is probably due to difference in growth rates in the stocks. As per the present results, morphometric data of Penaeus indicus differentiates Tuticorin-Madras, Cochin-Madras stocks completely and the resulting relationships among the stocks did not appear to resemble the relationships obtained from the biochemical electrophoretic data. But Cochin-Tuticorin geographical populations of P. indicus and Cochin-Bombay geographical populations of P. stylifera showed significant variation only for few morphological variables. Kirkpatrick and Selander (1979) found out speciation occurs in sympatric stocks of white fish with only minor changes in the allelic frequencies measured by electrophoresis.

Lester (1983) found out that different variables analysed in each species data set have quite distinct

correlations despite their appearance of morphological similarity among penaeid species. In the commercial mariculture operation of prawns any reduction of the size of the maturation tanks, hatchery tanks and growout ponds will affect behaviour and survival (Lester 1983), and consequently size.

Phenotypic differences observed between prawn species P. indicus samples of Tuticorin-Madras, Cochin-Madras, may be due to morphological differentiation in response to environmental factors during the ontogeny when these prawns enter the back waters and lakes for larval development as seen in snow crabs (Davidson et al, 1985). P. indicus can withstand wide range of salinity, especially in younger stages. To some extent the species is eurythermal, as seen from wide gradient of temperature of its natural habitats. Thus they are faced with relatively heterogeneous environments. The magnitude of larval exchange between areas would be effected by oceanographic patterns and proportional to the distance between areas and velocity of surface currents (Davidson et al, 1985).

As they grow larger they move to the sea and thus have a relatively homogeneous adult environments. Then tend to converge in their morphological attributes as seen in snow crab (Davidson et al, 1985).

Invertebrates such as crustacean generally exhibit little morphological change in relation to short-term changes in their environments. Their rigid exoskeleton often allows for more precise morphometric measurements compared with soft bodied vertebrates (Davidson et al, 1985). This proves that morphological comparison of crustacean population may provide useful evidence for delineating stocks. But it is often difficult to distinguish between genetic and environmental effects on phenotypic characters (Booke 1981). But majority of the phenotypic variability (Morphovariance) observed between areas would be due to the notion that structural genes evolution (measured by electrophoresis) proceeded independently at a different rate from evolution at more complex phenotypic levels (King and Wilson 1975, Wilson, Maxson and Sarich 1974; Wilson, Sarich and Maxson 1974). Besides these rate of protein evolution appears to be proportional to time (Ayala 1976; Carson 1976). This is probably the reason why some morpho variance is noticed in specimen from different areas, although biochemically they appear to belong to the same population.

SUMMARY

1. For the detailed analytical work different procedures were modified and methods standardised in order to get better results. For optimum resolution of different enzymes standardisation of methods indicated 10% acrylamide concentration giving best resolution for protein extracted from different tissues of the species of prawns under study.
2. The buffers employed for the separation of the enzymes Acid phosphatase, Alkaline phosphatase, Alcohol dehydrogenase, Aldehydeoxidase, Esterase, Alpha glycerophosphate dehydrogenase, Lactate dehydrogenase, Malate dehydrogenase, Malic enzyme, Octanol dehydrogenase, Peroxidase, 6-Phosphogluconate dehydrogenase, 1-Pyrroline dehydrogenase, Tetrazolium oxidase and Sorbitol dehydrogenase were Tris citrate Buffer pH 7; Tris citrate Buffer pH 7, Tris versene Borate Buffer pH 8, Tris glycine Buffer pH 8.3, Histidine pH 7 & Sodium citrate pH 7, Tris versene Borate pH 8, Tris citric acid pH 8.3 and Lithium hydroxide pH 8.26, Tris glycine buffer pH 8.3, Tris maleic acid Edta pH 7.6, Tris Maleic acid Buffer pH 7.6, Histidine pH 7 and Sodium citrate pH 7, Tris versene Borate pH 8, Tris versene Borate pH 8, Tris versene Borate pH 8 and Tris glycine Buffer pH 8.3 respectively.
3. The electrophoretic patterns of 15 different enzymes and their loci tested in different tissues, namely eye, hepatopancreas and muscle of two species of prawns P. indicus and P. stylifera has been studied for the first time.

4. Muscle myogen pattern of closely allied species of prawns were analysed to find out the interspecies genetic variation. Species of genus Metapenaeus, namely M. kutchensis, M. affinis, M. monoceros and M. brevicornis collected from Bombay waters showed characteristic bands of 7, 12, 9 & 9 respectively. Similarly species of genus Parapenaeopsis such as P. sculptilis, P. stylifera and P. hardwickii from Bombay had 9, 8 & 10 bands respectively.

5. Very closely allied species like Penaeus penicillatus and P. merguensis as well as P. japonicus, P. latisulcatus and P. canaliculatus were further subjected to ultra scanning and photography of the gels which showed distinct band nature useful for identifying the species.

6. The present ontogenetic observation in P. indicus shows that each larval stage of a species can be clearly identified on the basis of species-specific number of enzymatic protein bands. In cases where same number of bands exist in different stages the characteristic pattern of the bands would be useful in differentiating these stages.

7. Electrophoretic investigations of different isozymes in Penaeus indicus and Parapenaeopsis stylifera have enabled to detect seven polymorphic loci in P. indicus and six in P. stylifera, out of 23 and 22 loci analysed in the respective species.

8. The lack of goodness of fit as per Hardy-Weinberg equilibrium, in the distribution of different phenotypes in all the population of P. indicus and P. stylifera tested from samples from different places may be due to deficiency of heterozygotes and excess of homozygotes.
9. Genetic identity and genetic distance estimates following the analysis of ~~Nei~~ as well as Roger suggests that the population samples from four locations in the case of Penaeus indicus and the population samples from two location in the case of Parapenaeopsis stylifera are genetically similar.
10. There is little evidence to show that the prawns P. indicus and P. stylifera are subdivided into two or more genetic stocks. For management purpose all the population of these two species of prawns sampled from different locations, namely P. indicus from Cochin, Tuticorin, Madras and Waltair and P. stylifera from Cochin and Bombay appear to belong to a single unit biochemically.
11. The observation of apparent polymorphism in the enzyme octanol dehydrogenase alone in the Waltair samples of P. indicus would suggest the probable existence of an isolated population of the species there.

12. In P. stylifera out of the various enzymes analysed acid phosphatase alone showed some difference in the phenotypic distribution and allele frequency between Cochin and Bombay samples.

13. Statistical analysis of certain selected morphometric characters of sample specimen collected from Cochin and Tuticorin in the case of P. indicus and Cochin and Bombay in the case of P. stylifera exhibited very little significant variation, in conformity with the biochemical results.

14. Thus, as far as P. stylifera is concerned the populations at both Cochin and Bombay appear to be the same both biochemically and morphologically. Similar is the case with P. indicus of Cochin and Tuticorin. However, in the case of P. indicus some significant variation has been noticed in certain morphological features between Madras-Cochin and Madras-Tuticorin specimens, probably brought about by the differential growth due to different environmental features in relation to geographical situation.

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